

Nuclear industry goes public

RECENT years have demonstrated that, in Britain at least, if any of the components of the unholy government-industry-trades union triumvirate start making signs that they are taking a view on a matter, that matter is fast becoming an important issue. So when the *Financial Times* organises a conference on nuclear power—or, more precisely, “Nuclear Power and the Public Interest: the Implication for Business”—it’s a fair bet that energy, and specifically nuclear energy, is an item on the UK public agenda.

Evidence for this, of course, was around before last week’s conference. Mr Benn, the UK Energy Minister, saw to that (if the world’s energy problems hadn’t already done so) with his recent energy talk-in and his public debate six months ago on the reprocessing of nuclear fuel. But with evidence a-plenty offered at the conference to show that the future use of nuclear power in industrialised countries could not be escaped, short of a return to an agrarian economy or a drastic fall in living standards, the nuclear issue seemed more confined than usual.

Still, the problem of the long lead-times attaching to nuclear operations helps to make nuclear power the live issue it now is. The likelihood of a UK “energy gap” in the 1990s is disputed in some quarters and subject to uncertainties, but if it is to appear, and sooner rather than later, it means decisions on nuclear programmes are needed now, with a correspondingly bigger margin of doubt on all fronts. And where there are decisions to be made, there too will the issues be created.

Hardly surprising, then, that people in the nuclear industry should be talking, a little over-dramatically, of its very survival over the period of energy surplus. Hardly surprising, either, that the Department of Energy, which doesn’t yet doubt that the nuclear option must at least be retained, should become the object of attention for those seeking to influence the decisions which the “public” character of the nuclear issue now foreshadows.

The critical decision, on whether to go ahead with a demonstration fast breeder reactor (FBR), is expected sometime in the autumn. Unexpected, if heavily qualified, support for a demonstration FBR came at the conference from the Chairman of the Royal Commission on Environmental Pollution, Sir Brian Flowers (now looking at the effect of nuclear power), and from Leslie Grainger of the National Coal Board. Great

uncertainty was again expressed, however, about the fate of the Steam Generating Heavy Water Reactor (SGHWR).

On less specific though no less important aspects of nuclear power, grave doubts were expressed, and brave responses offered: about the disposal of radioactive wastes after reprocessing (confidence was expressed about glassification techniques, and about burial in deep holes or under the ocean bed); about health and safety in the nuclear industry (favourable comparisons were drawn with other industries, and especially coal); about proliferation through international nuclear trade (to stop this, claimed one businessman, would be to encourage countries to develop their own technology over a longer period); about the chances of a nuclear accident (all necessary precautions were taken) and nuclear terrorism (the chances of a successful action were minimal); and about the economic facts of nuclear power (which, like all economic “facts”, it became apparent, translate only as interpretations). To emerge with the right questions seemed more important in the end than having pat answers.

This was less true of other controversial questions which were broached about the nuclear issue. What should the role of the state be in the nuclear industry, if not in encouraging private enterprise in its task? How was a stream of steady orders to be secured to tide the UK industry over the coming years, if not by breaking into the export business with a marketable reactor? How was any progress to be made without international cooperation, specifically with Britain’s European partners? Disappointingly, these questions, for most delegates, contained their own answers.

Not that the unique long term aspects of nuclear power (“making decisions for future generations”) weren’t acknowledged. But views on nuclear power for the most part revealed differences of detail rather than principle. Although the differences weren’t insubstantial for that, there was unfortunately no real voice posing the obvious question—namely, who or what is the anti-nuclear lobby in Britain? (Everyone knows the answer to the analogous American question.)

This latest conference on the subject indicated that the British nuclear industry is finding it necessary to change its public position by actually adopting one. But if nuclear power is really on the public agenda, there are still more fundamental issues to be discussed, if only to be confirmed as resolved. □



The weather a week ahead

Ernest Knighting describes the establishment of the European Centre for Medium Range Weather Forecasts

RELIABLE weather forecasts for a week or so ahead would be of great economic value; almost every industry, including agriculture, transport, building, shipping, fuel and power, would benefit in the planning of operations and, of course, the general public would be better able to plan outdoor activities. And if, as has been estimated, the benefit to Europe would be more than £100 million a year at 1970 values, it is well worth setting up an organisation to conduct research into problems associated with medium range forecasting, and eventually to provide operational forecasts.

The resources of skilled manpower and equipment required for such a task, however, exceed those normally available at national level. This makes an international co-operative effort necessary, and in October 1973 sixteen European nations signed a Convention establishing the European Centre for Medium Range Weather Forecasts. By November of last year a sufficient number of nations had ratified the Convention for the Centre to come into being. Belgium, Denmark, West Germany, Spain, France, Ireland, Yugoslavia, the Netherlands, Austria, Portugal, Switzerland, Finland, Sweden, Turkey and the UK have ratified, while both Greece and Italy are expected to ratify shortly.

The Centre's headquarters (to be erected by the UK Government) will be at Shinfield Park, Reading, England; temporary accommodation has meanwhile been provided at Bracknell. The Centre will have a staff of about 140, including about 70 graduates, mostly in the research and operations department. The present staff, numbering about 40, reflects the international character of the Centre, with nine member nations being represented. In addition there will be opportunities for visiting scientists to work at the Centre.

The Director, the well-known Danish meteorologist, Professor Aksel Wiin-Nielsen, was formerly Head of the Department of Atmospheric and Oceanic Sciences at the University of Michigan; he, together with a small planning staff, laid the foundations of the Centre's development so that at its official birth it was already well-advanced in its scientific and operational planning. When the Centre is fully operational it is expected to have a yearly budget of about £6 million; the cost will be met by the member nations, contributions being scaled according to the gross national product of each nation.

The central premise of the develop-

ment of these operational forecasts is that the atmosphere may be regarded as a compressible fluid, its behaviour being described by the Navier-Stokes equation and the thermodynamic equations concerned with sources, sinks and the transfers of energy. Considerable progress has been made in the last two decades in using these equations for two distinct objectives. The first is for short-range weather forecasts for a day or two ahead, and it is now common for national services, especially those in extra-tropical latitudes, to base their short-range forecasts on the numerical integration of the equations. The problem is the prediction with considerable accuracy of the motion and development of weather-bearing systems, which have a lifetime of a week or so; it requires a fairly precise knowledge of the state of the atmosphere at the instant from which the forecast proceeds, while some slow acting physical processes may be neglected.

The second objective is to determine why the atmosphere has its general climatic structure and to throw light on climatic change. In this research the equations are integrated for perhaps a hundred days or more rather than for the one or two days of the short-range forecast. During this period many generations of weather-bearing systems undergo their life history and the emphasis is no longer on the initial detail but on the careful representation of the physical processes that are thought to be important for the atmospheric development over a long time, such as incoming and outgoing radiation, the transfer of energy at the earth's surface and so on.

Forecasting for up to ten days ahead presents a more difficult problem because both the initial details and the careful accounting for the physics have to be taken into consideration. The detail must be considered because the evolution of the atmospheric systems in existence initially has a considerable effect over the period; indeed, some of the systems may persist, although modified in form and position, for most or the whole of it. The changes caused by the slow acting physical processes can also be profound during the period; the effect of a change in sea surface temperature, for example, due perhaps to up-welling, can be remarkable. Over a few days the development of the weather bearing systems over Europe and the Atlantic can be affected by what is happening very far away, and forecasts for ten days ahead need to be global, taking



Professor Wiin-Nielsen, ECMRWF Director

into account the developments in the Southern Hemisphere, which are less well defined, as well as in the Northern Hemisphere.

The equations describing the evolution of atmospheric motions are highly non-linear and whether any useful analytic solutions will ever be obtained seems doubtful. Certainly the only known methods of solution are numerical, and the arithmetic burden is enormous: operational numerical forecasts for a couple of days ahead require the use of large-scale computing facilities with speeds of operation of about 10 million instructions per second; computations associated with operational forecasting up to ten days ahead call for facilities about five times as fast.

If the Centre is to achieve the difficult, perhaps impossible, target it has set itself—namely to be in a position to provide daily operational forecasts before the end of 1980—there must be scientific research and development on three fronts. The first concerns the acquisition of the right daily data, its verification and reduction to a suitable form. This problem has become very complicated because a great number of meteorological data, especially those from satellites, are observed continuously and require special methods of assimilation if they are to be used in the forecasting process. The second concerns the important physical processes which often cannot be represented directly in the computations because the arithmetic has to be carried out for discrete grid points about 100 km apart. Atmospheric processes smaller in size than this can only be represented statistically in the computations. Many of the important energy transfers—those due to vertical instability and the development of cumulo-

nimbus clouds, for example—are sub-grid scale in size and their important contribution to the working of the atmosphere needs investigating. The third concerns the selection of the mathematical methods of integration, where there will be a delicate balance between accuracy and economy.

An additional complication is the problem of predictability which is currently exercising meteorologists. It is the problem of determining for how long ahead useful forecasts can be made. It is generally agreed that the limit is less than one month and more than five or six days, using current mathematical models and the meteorological observations presently available. Experimental determination of the limit by integration of the model equation is very difficult because a few integrations cannot give results representative of so variable a fluid as the atmosphere, while each integration predicting for, say, 14–20 days, is extremely costly. Experiments carried out up to

date show that there is value in the forecasts up to ten days and this is confirmed by the few integrations carried out at the Centre. The Centre will co-operate as fully as it can in the First GARP Global Experiment, which is designed, among other objectives, to determine the limit of predictability.

With the aid of computer programmes for atmospheric models provided by the Geophysical Fluid Dynamics Laboratory of Princeton, the Department of Meteorology, UCLA, and the UK Meteorological Office, this research has already started. Ten days forecasts have already been made with these models, using both a CDC 6600 computer installed for the Centre's use by CDL in Bracknell and the UK Meteorological Office computer complex; their production is regarded as a familiarisation exercise.

A considerable effort must also be devoted to the selection and installation of suitable computing facilities; speeds of 50 million instructions per

second are only just being developed and the selection of an integrated system capable of carrying out the data processing and the number-crunching on an operational basis will certainly present difficulties. Of course, the system must be available before 1980 since the Centre will need to develop its operational programmes on it.

Europe, through the member nations, has created the first scientific centre for the study and preparation of medium range weather forecasts. The international meteorological community has expressed great interest in this new venture with offers of help and co-operation. Already a cooperative agreement has been signed with the World Meteorological Organisation and the provision of computer programmes is one example of the help that has been made available. Nevertheless the Director and his staff face a difficult task in attempting to build up a new international scientific centre in a time of recession. □

USA

Science vs. the public

Colin Norman reports from Cambridge, Massachusetts, where attempts are being made to restrict recombinant DNA research.

IN a move which establishes a virtually unprecedented degree of community control over an area of scientific research, the city council of Cambridge, Massachusetts, last week passed a resolution calling for a three-month moratorium on certain types of recombinant DNA research at Harvard and Massachusetts Institute of Technology. Though the moratorium will have little immediate impact, since experiments of the types outlawed by the council are not yet being conducted at either university, the council's actions could have broad and lasting implications.

While the moratorium is in effect, a special review board, also established by the council last week, will draw up recommendations to control recombinant DNA research in Cambridge. In addition the board has been given the astonishing task of reviewing all laboratory experiments in the city, to ensure that none of them poses a threat to public health.

The council's actions are the result of a bitter clash between groups of scientists, city officials and Cambridge residents over plans to conduct recombinant DNA research at Harvard and MIT. The dispute, which centres on the question of whether or not such experiments can be carried out safely, has been a bloody battle which has provided ample evidence of public

distrust of scientists and which has further damaged the already abysmal relationships between the city and its two famous universities.

In a sense, the clash has also provided the first real test of public acceptability of federal safety guidelines governing recombinant DNA research issued last month by the National Institutes of Health (NIH) (see *Nature*, July 1). The guidelines were developed mostly by scientists, and have not previously been subjected to the intense public scrutiny now being lavished on them in Cambridge. Those who support the guidelines—and they include the vast majority of the scientific community—can take little comfort from the public debate in Cambridge. Moreover, many scientists are worried that similar clashes could break out elsewhere, a development which the Mayor of Cambridge has been doing his best to promote by trying to interest his fellow mayors in the issue.

The hostilities stem originally from a proposal, put forward two years ago by a group of scientists headed by Mark Ptashne, to convert some rooms on the fourth floor of the Harvard Biological Laboratories into a special safety facility. Designed primarily for experiments with the animal virus SV40—experiments which are frequently carried out in other institutions under much less stringent safety conditions—the facility became a major focus of controversy when the plans were modified to include space for some recombinant DNA work.

At present, Harvard researchers are restricted to experiments, judged by those who wrote the guidelines to entail little risk, which do not require special safety facilities. According to Matthew Meselson, Chairman of the Department of Biochemistry and Molecular Biology, about 30 people at Harvard are now working, at various levels, on such experiments. Construction of the safety laboratory—a moderate containment, or P3, facility under the NIH guidelines—would allow many other types of experiments to be undertaken at Harvard.

Opposition to the facility came first from scientists at Harvard. It took two forms. Some argued that although recombinant DNA research is an exciting and potentially revolutionary area of research which should be conducted at a major research institute like Harvard, the building housing the biological laboratories is unsuitable for a safety facility. It is old, infested with ants and cockroaches, and situated in a densely populated area. The second, more fundamental objection came from scientists who argued that recombinant DNA research is inherently so risky that virtually all such experiments should be confined initially to one or two specially equipped national facilities, or be outlawed entirely, until the hazards are better defined.

In spite of the intensity of the opposition construction of the facility has been approved in principle by a special biohazards committee, by Harvard's top-level committee on research policy, and by the Dean of Arts and Sciences. Final plans for the lab are now being drafted.

The internal dispute at the university spilled over into the city and mushroomed into a major political issue early last month when the matter was reported at length in the *Boston Phoenix*, a weekly area newspaper. At that point, Cambridge city Mayor Alfred E. Vellucci stepped in. An irascible, colourful, shrewd politician with 26 years' service on the council, Vellucci is no friend of Harvard or MIT. In fact, the strained relationships between town and gown in the city provide an important backdrop to the clash over recombinant DNA research in the city.

A major source of irritation is the fact that the two large, wealthy universities are situated in a predominantly industrial community and are exempt from paying many city taxes. There have also been problems over pressure on city housing—"If it gets any worse", Vellucci said in an interview last week, "we'll have to knock down some buildings for somewhere for people to live". A good example of town-gown friction came a few years ago when the city council passed a resolution, introduced by Vellucci, to pave over Harvard Yard and turn it into a parking lot. (The resolution, according to Vellucci, successfully spurred the universities into building more student parking facilities.)

Vellucci's dignity was pricked when he read in the *Phoenix* that Harvard scientists were planning to conduct reportedly dangerous experiments without first consulting city officials. And his belief in the dangers of the experiments was heightened by a visit from George Wald, a Nobel prizewinner and a vigorous opponent of the plans for the laboratory. Vellucci called a council meeting for June 23—ironically the day on which NIH issued its guidelines—to discuss the matter. The meeting provided a forum for a bitter clash between mostly radical scientists and their more establishment colleagues, with council members often bewildered and Mayor Vellucci enjoying the publicity, and especially enjoying the opportunity to haul Harvard intellectuals over the coals.

At one point in the proceedings, Vellucci particularly chastised Daniel Branton, chairman of the biohazards committee, for making little attempt to bring city officials into the discussions of plans for the safety laboratory. Branton protested that he had, but Vellucci scored a major debating point.

During the meeting, Vellucci proposed a resolution calling for a two-year moratorium on *all* recombinant DNA research in the city—even those experiments already under way which are judged to entail little risk. No vote was taken at that meeting, however, and it was adjourned after some

five hours of bitter exchanges.

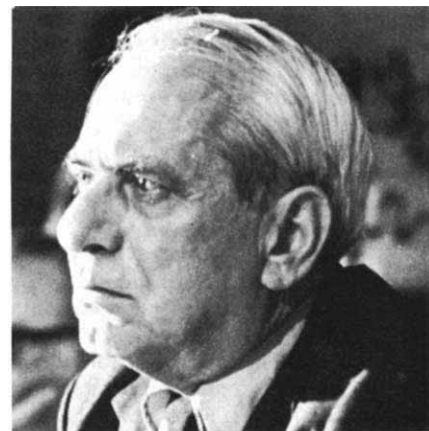
The next day, Vellucci issued a public statement declaring that the meeting "has made it clear to me that a 'cooling off' period is necessary in order for the mayor and the council to deal with this matter in a calm and rational way". He then softened his proposal for a two-year moratorium, calling instead for a three-month 'good faith' halt to the research "so that all concerned can properly review relevant testimony. He later offered another proposal to establish a review board to look into the issue and to examine other research programs at Harvard and MIT to see whether any pose a potential public health risk. A second council meeting was called on July 7 to resume testimony and to discuss the resolutions.

In the fortnight between the meetings, some councillors and the mayor spent a good deal of time contacting, and being contacted by, scientists. As a result, Vellucci announced to the meeting last week that he had "learned enough about recombinant DNA molecules in the last two weeks to take on all the Nobel prizewinners in the city of Cambridge". He has certainly taken on most of them.

The meeting began with a demonstration of techniques used in microbiology, for the councillors' edification. Meselson then described the formulation of the NIH guidelines, noting that in each new draft they were made more strict. And William Gartland, Director of the newly created NIH Recombinant DNA Activity Unit, described the guidelines themselves, pointing out in particular the contrast with the situation in the United Kingdom, where the whole business of setting regulations on the research is taking place behind closed doors.

At that stage, Councillor David Clem, a young, articulate council member who has obviously done his homework on the issue, proposed a number of amendments to Vellucci's resolutions. The most important change was an amendment to limit the moratorium to research requiring P3 and P4 facilities, according to the NIH guidelines. That amendment was accepted without debate, even though it meant the difference between stopping several experiments and stopping nothing.

Harvard has no P3 or P4 facility, so no such experiments are going on there. MIT, however, has a P3 facility in the Center for Cancer Research, but no P3 level experiments have yet been initiated. One experiment is being planned at the facility by Dr Philip Sharp, however. He said last week that he has been holding off on the research—which would involve inserting mammalian genes into *E. coli*—until the



Photos: Noëleen Chedd

Mayor Alfred Vellucci (top) and (below) Daniel Branton

NIH guidelines had been published, and that it would take at least three months to get the experiment approved and under way.

Another council member, Sandra Graham, then proposed that the moratorium should be extended from three months to six. After some debate, the amendment was defeated by six votes to two. Vellucci then proposed that the moratorium should be extended to two years if no agreement has been reached at the end of three months. That was also defeated by six votes to two. At that point, the resolution with Clem's amendment was approved by five votes to four.

The second resolution, establishing a Cambridge Laboratory Experimentation Review Board, was then voted on, and similarly approved by five votes to four.

With the amended resolutions approved further testimony on them became superfluous but it was taken anyway—until 12.45 am—with the council chamber packed with scientists and with people from both sides heatedly stating their views.

A number of interviews with scientists the day after the meeting demonstrated that both sides are relatively pleased with the outcome so far. Proponents of the research are happy that the moratorium will do no damage to current research and that it

is much less restrictive than the original resolutions. And opponents of the research are pleased that the vote has established the principle of community control over scientific research.

There is, however, considerable unease within the scientific community, and with good reason. The council meeting secured a precarious cease-fire, which could easily erupt into renewed hostilities. An important factor in what happens next will be the work of the experimentation review board. Its terms of reference are broad and hazy, and its members are yet to be appointed. One potential member is George Wald, who was asked by Vellucci last week whether he would serve if asked and replied that he would be "happy and honoured to do so". Vellucci said after the meeting that the board would contain both supporters and opponents of the research and that some city residents would also be included. If necessary, he said, he would lock them all into a room and not let them out until they have come to an agreement.

Creation of the board has at least taken the council out of its uncomfortable position in the middle of the dispute. But if the board has not reached agreement at the end of three months, the council will be thrown right back in and there would then be a possibility that the moratorium would be lengthened and, perhaps, broadened. Alternatively, if the board vigorously pursues its mandate to look into all laboratory work in Cambridge, there is a danger that it could turn into a public witch hunt. "We will be keeping our fingers crossed in the next three months," says Richard Leahy, a dean in the Faculty of Arts and Sciences.

On the other hand, creation of the review board at least establishes a long-needed channel of communication between the university scientists and the city. It could provide an important vehicle for informing and educating the people of Cambridge about recombinant DNA research and about the process which led to adoption of the NIH guidelines. It is conceivable that it could even help smooth some of

the causes of town-gown friction.

Another possibility which is clearly worrying people far removed from Cambridge is that similar clashes could erupt in other cities. In that respect, Vellucci has been active. Last month he attended a meeting of the United States Conference of Mayors armed with a resolution stating that "no university or institution may begin recombinant DNA experiments without first notifying the mayor of the city or town in which the experimentation will take place. Thereupon, the mayor and legislative body shall call a public hearing so that the public may be an active participant in the decision-making process". The resolution was referred to a committee for consideration, and Vellucci said last week that he intends to press the matter at future meetings.

It is possible that the dispute in Cambridge will now quietly die away. But with so many articulate and politically motivated scientists opposed to the research there, the prospects at this stage are far from certain. The next few months will be critical. □

EEC

JRC programme awaits approval

The Commission of the EEC has circulated final proposals for the next multiannual research programme of the Joint Research Centre. These now await ratification from the Council of Ministers. Chris Sherwell reports

THE multiannual research programme of the European Community's Joint Research Centre (JRC) for the four years beginning in January 1977, the final detailed proposals for which were submitted by the Commission to the Council of Ministers some two weeks ahead of the Council's May 24 meeting, may not be approved before September, even though preliminary discussions of the overall framework for the Community's 1977 budget are due to start later this month.

The Council decided at the May meeting to consult both the European Parliament and the Economic and Social Committee, even though there is a clear need for the JRC's budgetary requirements for 1977 to be entered into the budget for the coming financial year. The European Parliament is being urged to deliver its opinion no later than its part-session in September, which is about the time when the Finance Ministers are expected to be reaching more concrete budgetary decisions.

The Commission's proposed programme represents the outcome of

many months of behind-the-scenes deliberation, including broad consultation with research and administrative staff. Last October the Commission sent to the Council its "Overall Concept" for the programme, setting out guidelines for the JRC's future role, and this the Council discussed at an inconclusive December meeting along with a document on a joint research and development policy. Other discussions took place in February, and the General Advisory Committee considered the draft programme in March and the Scientific and Technical Committee considered it in April.

The Commission has already stated that in framing the new programme it took into account "criticism made in the European Parliament and elsewhere" that the JRC "had engaged in too many 'half-baked' projects which it had not the resources to complete"—criticism which arose, it said, "in part from the failure of the member states to provide the Centre with effective guidelines".

Details of the final proposals now under consideration are shown in the accompanying table, but do not include the installation of the JET fusion project, which the Commission wants at Ispra, since a decision on the matter is still awaited.

In its final proposals the Commission points out, some would say rather obviously, that among the

guiding principles of the programme, primary consideration was given to the Community's interests in science and technology. From its discussion of these principles and of the JRC's role, however, a few important points emerge, namely, the priority being accorded to energy and environment questions, the stress on a Community public service role for the JRC (that is, making equipment, know-how, products and services available), and the concentration of activities and research potential on a small number of projects. There is also emphasis on the "rolling plan" character of the proposed programme, and on management and staff aspects.

The three-year rolling system, proposed in order to allow a greater degree of adaptability, is quite simple. The four-year programme would be submitted during the third year, when the Council would adopt a new four-year programme in which the last year of the initial programme would become the first year of the new programme. The new programme would itself be submitted for revision during its third year, when a decision would be taken on the following programme. In the absence of decisions, current programmes would be continued according to the Council's original decisions.

Regarding the financial requirement over the forthcoming four-year period of 374.4 million units of account (£156 million), the Commission says the pattern of investment expenditure envisaged means in practice that it will not

be divided into equal annual parts; rather, it is expected that the annual budgets will actually show a decrease over the four years.

The impact of proposals regarding researchers attached to the JRC has statistical consequences to which the Commission draws attention. Its concern over their mobility is reflected in

the proposal, approved broadly by the European Parliament and still under examination by the Council (the Commission urges that it be adopted as soon as possible), for a different manner of payment; a new "research staff" concept is also adopted which stretches beyond existing "first line staff" to include associated technical

support staff.

Thus, as the document goes on to point out, if the "research staff" number and programme expenditure are taken together as a measure of the distribution of effort within the JRC, programmes I, II and III (which can be broadly classed as "nuclear safety") take up 48% of the total, future

The Multiannual Research Programme of the JRC proposed by the European Commission

Programme	Projects in programme	Total	Personnel		Specific Programme Expenditures (muc)	Staff Costs research ² (muc)	Global Supporting Costs (muc)	Total Costs (muc)
			Research Staff	Others ¹				
I Reactor Safety	Reliability and risk assessment; Light water reactors loss of coolant accidents; Liquid metal fast breeder subassembly thermohydraulics; Fuel coolant interactions and core melt-down; Dynamic structure loading and response; Structural failure prevention	440	239	201	16.52	38.38	37.23	92.13
II Plutonium fuels and actinide research	Utilisation limits on plutonium fuels; Plutonium and actinide aspects of the safety of the nuclear fuel cycle; Actinide research	209	121	88	6.6	15.3	19.58	41.48
III Management of nuclear materials and radioactive waste	Evaluation of long term hazards of radioactive waste disposal; Chemical separation and nuclear transmutation of actinides; Fuel materials management; Reactor component decontamination studies	161	97	64	4.36	15.78	14.24	34.38
IV Solar energy	Thermal conversion in housing; construction of European installation to simulate solar irradiation; orientative research on advanced applications of solar energy	57	35	22	4.86	5.67	6.74	17.27
V Hydrogen	Thermochemical processes for water decomposition; heat source coupling	78	50	28	2.4	8.14	5.92	16.46
VI Conceptual studies on thermonuclear fusion	Participation in conceptual study of an experimental power reactor in connection with Euratom-CNEN association at Frascati (blanket neutronics, heat transfer and energy conversion systems etc)	14	8	6	0.28	1.28	0.99	2.55
VII High temperature materials	Industrial needs; effect of operational environment on mechanical properties; failure modes in applications; relationship of structure and properties	51	36	15	2.21	4.44	2.54	9.19
VIII Environment and resources	Atmosphere; water; chemicals; renewable (agricultural) resources	188	115	73	5.01	18.73	17.59	41.33
IX Measurements, standards and reference techniques	Measurement of nuclear data; nuclear and non-nuclear reference methods and techniques; scientific support to the services of the Commission and Secretariat of Community Bureau of Reference	286	181	105	8.185	25.51	25.995	59.69
X Services and support activities	Exploitation of high flux reactor; informatics; education and training; safeguards; technical evaluations in support to the Commission	221	116	105	26.505	14.94	18.465	59.91
Totals		1,705 ³	998	707	76.93	148.17	149.29	374.39

Units of account based on value at 1 January 1977.

¹ Does not include 471 local agents not presently classed as personnel because they are paid from funds allocated yearly from the appropriate section of the budget.

² Includes laboratory equipment costs.

³ To this total must be added 183 people relating to the ESSOR reactor.

energies (programmes IV, V, VI and VII) take up 12%, environment and resources 10%, reference work 17% and support 13%.

This as the Commission says, means that effectively 70% of the JRC's effort will be devoted to "energy and the environment", with much of the effort in energy concerned with nuclear

safety. The Commission points out elsewhere that the proposals also achieve a better (60 : 40) balance in the ratio of payroll costs (214.6 muc) to operating expenses (159.8 muc).

The new programme, provided it is adopted in time, will follow on the existing multiannual programme, adopted in 1973, being carried out in

the four JRC establishments at Geel (Belgium), Ispra (Italy), Karlsruhe (West Germany), and Petten (Holland). The large body of the work will be at Ispra. Karlsruhe will take the lead in plutonium fuels and actinide research, Petten will work on high temperature materials, and Geel will coordinate the work on reference methods. □

BRAZIL

● Brazilian engineers have now succeeded in modifying an automobile engine to run on pure alcohol. This could be significant in solving energy problems here, in view of the fact that Brazil imports more than 80% of the crude oil it uses for making petrol. The experimental engine, installed in a Brazilian-made Dodge sedan and demonstrated to government officials in Brasilia, achieved 23 miles per gallon—roughly the same as that model gets with a gasoline engine—and had no trouble keeping up a steady 55 miles per hour in a highway test. The alcohol engine also released far fewer pollutants in its exhaust than a standard engine. Brazil's commerce and industry minister, Severo Gomes, was impressed by the demonstration. He said that if an alcohol engine is produced in volume here, it will be put first in buses and taxis in big cities.

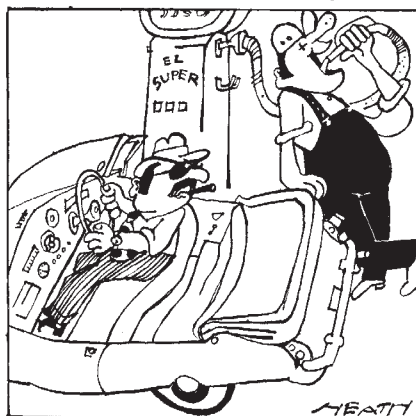
Brazil has the potential to be self-sufficient in alcohol fuel. It is the world's biggest cane sugar grower (sugar is the country's current prime source of alcohol), and it has unmeasured quantities of cassava, a plant whose root provides a basic food for millions of poor farmers and which local scientists say can also be made to yield commercially usable alcohol.

The government's oil refining monopoly, Petrobras, currently dilutes its gasoline by 20% with alcohol, to make imported crude oil stretch further.

● Sao Paulo, Brazil's biggest metropolis (city population: 7 million; including the suburbs: 10 million), also has South America's biggest air pollution problem. A combination of thermal inversion, industrial smoke and automobile exhaust gives Sao Paulo the look of London in the days before anti-pollution measures went into effect in Britain. The air quality in Sao Paulo is often so bad that department stores take out newspaper advertisements for filter masks.

But now local health officials have persuaded the mayor's office and the police to cooperate in a plan called "Operation Winter", which will be put into effect on badly polluted days during the South American winter

(June–September), when pollution-trapping temperature inversion is at its worst in Sao Paulo. The operation has three stages: a warning level, an alert level, and an emergency level when local authorities will prohibit the use of petrol and diesel-powered



vehicles, shut down all factories and prohibit all burning.

This type of action has never before been contemplated in Brazil, where the official line has been that pollution is the price a developing country must pay if it wishes to progress since time spent on its control is time wasted.

● Brazil has been complimented for its progressive laws protecting wildlife and, at the same time, criticised for its inability to enforce them. This is especially true in the vast, underpopulated Amazon region, where it is virtually impossible to control illegal trafficking of protected species of birds and animals.

Occasionally, though, the authorities get results. Police in Rio followed up a recent tip that dogs and cats in a residential neighbourhood had been mysteriously disappearing, and this led them to what apparently is a large-scale contraband ring for exporting Brazilian Amazon creatures such as monkeys, parrots, parakeets, tucans and canaries.

Four persons were arrested on suspicion of running an illegal animal trade to Europe, North America and Japan. The police found hundreds of caged birds and monkeys awaiting shipment out of the country. They also found many dead animals which did not survive flights from the

Amazon city of Belem to Rio. The operators of the smuggling ring apparently had been using local dogs and cats to make food for the captured Amazon animals.

In similar actions, police in Sao Paulo and Fortaleza recently broke up illegal trafficking in songbirds which are protected by wildlife laws. There is a big local demand for many varieties of these popular birds in Brazil, and breeders and sellers seem willing to run the risks to make a profit.

● In another instance of Brazil's finally applying a law that had seemingly existed only on paper before, naval authorities in the north-eastern city of Salvador slapped a fine equivalent to \$25,000 on a local titanium company after a pipe broke at its factory and sent sulphuric acid and ferrous sulphate spilling on to Atlantic Ocean beaches, leaving a brownish stain several miles long in the water. Among the beaches hit was Arembepe, which often is played up in tourist brochures as a Brazilian "unpolluted paradise".

● Brazil's biggest lagoon, Lagoa dos Patos, which is near the country's far southern tip, is becoming so polluted that fish and shrimp are dying *en masse*, and even some farm animals have died of poisoning after drinking water from it. Scientists at the Rio Grande do Sul State Center for Toxicological Studies are not sure, but they think the cause is a sharp increase in the use of DDT and other insecticides on nearby farms. Soybean and wheat production in Rio Grande do Sul have gone up dramatically in recent years, and farmers there traditionally have used large amounts of pesticides.

Fishermen around Lagoa dos Patos say their annual catch in the 3,800-square-mile lagoon is going down steadily. And bird watchers in the region have noticed that the once-common marsh cardinal no longer is seen and that the Patagonian duck does not stop at the lagoon any more on its winter flight north from Argentina.

Bruce Handler
Rio de Janeiro

SWEDEN

Nuclear fuel report out

After three and a half years of study, the Swedish Commission on Spent Nuclear Fuel and Radioactive Waste recently produced its final report. Wendy Barnaby sent this view from Stockholm.

"EVEN today's techniques offer satisfactory possibilities for the handling and storage of spent nuclear fuel and radioactive waste." Such is the assessment of the Swedish Commission on Spent Nuclear Fuel and Radioactive Waste, whose task was to study the management of low-, medium- and high-level radioactive wastes—that is, not only those from nuclear power plants, but also those from research institutions, hospitals and industry.

The timing of the report was no doubt planned to support the government's pro-nuclear power stance in the current general election campaign, in which the ruling Social Democrats are under heavy pressure from the opposition—part of which is adamantly opposed to nuclear power. Thus the report's claim—that its "central demand" was that all steps in the handling of radioactive waste had to be formulated with regard to the protection of people and their environment now and in the future—sounds suspiciously like an attempt to pull the carpet out from underneath the opposition, whose criticism of the safety and environmental aspects of nuclear power has mobilised an unusually large following. The latest opinion polls show that both government and opposition parties have lost support for their nuclear energy platforms since last January and that, while the opposition's policy is still more popular than the government's, more people are doubting the wisdom of either. This result will obviously stimulate each camp to even greater propaganda efforts than before, and the Commission's report will undoubtedly be one of the government's main weapons.

The report has not definitely pronounced on the fate of Sweden's spent fuel. "We recommend that this decision should be taken in 1981 or 1982", says the Commission's Secretary, Mr Philip Moding. The options are to continue sending spent fuel abroad for reprocessing, or to deal with it in Sweden reprocessed or not. In the long term, sending it abroad is no satisfactory solution. Under an agreement that will last until 1980, spent fuel from the Oskarshamn plant is being sent for reprocessing to Windscale in England. Negotiations are presently going on between the Swedish

Nuclear Fuel Supply Company (SKBF) and United Reprocessors, jointly owned by British, French and German interests, for the spent fuel that will be taken from plants already in operation at Ringhals, Forsmark and Barsebäck. A preliminary understanding has been reached but nothing has been signed; and the hoped-for agreement would probably only last until 1980 anyway. "It will be necessary for us to build an intermediate storage capacity for spent fuel in any case", says the Director of SKBF and an expert adviser to the Commission, Dr Erik Svenke, "because even if we continue to send spent fuel abroad, the reprocessor will have the right to return the waste to Sweden and we would then have to store it here."

The report urges the acceleration of research into ways of handling spent fuel domestically without reprocessing. How seriously this proposition is meant is hard to say. A domestic solution will in the long term certainly be preferred to the difficulties and uncertainties of sending it abroad, especially as Sweden's neutrality makes the country seek as much independence as possible in questions related to its national security; and it seems almost certain that a reprocessing plant, with its attendant business opportunities and relative simplification of storage of remaining waste, will then seem the best solution. Dr Svenke is convinced that Sweden will have to build a reprocessing plant at some time. "A country with a civil nuclear programme has a responsibility to take care of its waste", he says.

According to the report, any decision to build such a plant should go hand in hand with one to construct a fuel fabrication plant, to ensure that both plutonium and uranium are re-used as fuel. It estimates that a reprocessing plant with a capacity of 800 tons of uranium a year (and offering employment for 1,000 people) would take about 13 years to build. The Social Democrats' plans for Sweden's nuclear future envisage 13 reactors in operation by 1985. So far, five are running and five others are under construction. When all are operating, the country's total net nuclear power capacity will be about 10 GWe and, it is estimated, the reactors will produce about 300 tons of spent fuel a year. The reprocessing plant's excess capacity would be intended for other countries' waste. The Nordic countries would be obvious participants, although neither Denmark nor Norway has yet decided to build nuclear power plants. Finland, however, had three under construction

at the beginning of this year, and it is expected that they will produce about 2.2 GWe by 1985.

The method of disposal recommended by the Commission is to melt the highly radioactive waste in glass and deposit the medium- and low-level waste in concrete, then store it all in vertical tunnels drilled out of bedrock at a depth of 300–400 metres. Studies made by the Geological Survey of Sweden have pronounced bedrock conditions stable and suitable for such storage. "It is very important that the Commission has demonstrated that the terminal storage problem can be solved in Swedish bedrock", says Dr Svenke. "It signifies that the fuel cycle can in fact be closed."

The President of Sweden's Friends of the Earth group, Lennart Daléus, however, is not so optimistic. "What the Commission is telling us", he maintains, "is that with current techniques and those they hope will be developed in the future, the problems of high-level radioactive waste disposal can be solved. But in fact this is more a hope than a technical certainty." He also raises broader issues which he says the report should have considered. What are the social and moral implications of undertaking to secure radioactivity for hundreds of years? How can we possibly be sure that future political regimes will be willing or able to take care of our waste? Dr Svenke does not think these issues are relevant. "The terminal storage of radioactive waste", he says, "is surely a technical problem. It does not have the same dimensions as other aspects of the question: the relationship between the spread of nuclear energy and the proliferation of nuclear weapons, for example"—although he is apparently prepared to take the risk of proliferation.

The report gives only a brief mention to the problems of terrorism and theft of plutonium, and it is noteworthy that one of the parliamentary members of the Commission, John Takman, felt obliged to publish an addendum to the report expressing his regret that the Commission had not taken more account of the relationship between nuclear energy and nuclear weapons. If the more pessimistic experts are proved right, and the spread of civil nuclear energy programmes does result in nuclear-weapon proliferation which does in turn produce global nuclear catastrophe, all the present arguments will be shown to be ironically irrelevant.

Such predictions, however, are not the stuff electoral victories are made of. Sweden's nuclear future will have to wait for clarification until September, when the results are known. □

ISRAEL

● Some unfashionable opinions on science and defence were expressed in Israel by Sir John Kendrew, now Director-General of the European Molecular Biology Laboratory in Heidelberg, when he spoke at a symposium marking the 60th birthday of Israel's scientist-President, Professor Ephraim Katzir. Sir John pointed out that Israeli scientists, living in a nation which had fought several wars of national survival, faced little moral conflict when it came to doing defence research. British scientists, in contrast, found such research "boring and disreputable."

"We now tacitly assume, in my opinion contrary to the evidence, that defence research is no longer necessary, that the Western countries will probably never again be at war. Our younger scientists don't want to get involved, and as a result our defence committees are manned by greybeards". Given the imperfections of the world we live in, Kendrew added, "I think that defence research is necessary still, even though it might not be as obvious as it was during the Second World War when I was masquerading as an Air Force officer and Crick as a Naval officer".

Kendrew expressed scepticism as to whether international agreements for arms control could be enforced, a scepticism which was not shared by Professor Carl Goran Heden of Stockholm's Karolinska Institute, who also participated in the symposium. As he stated in his multi-volume report on biological warfare that was prepared for the Stockholm International Peace Institute (SIPRI), Heden thinks that the major powers will make and keep agreements. He admits, however, that small groups of fanatics might take the law into their own hands, which emphasises the need for an international criminal code to deal with such groups.

● If Israeli lives are to be saved, military research may be less important than research on traffic accidents, which claim far more victims than do the fields of battle. Israeli motorists kill off one another, as well as pedestrians, at a rate practically unequalled elsewhere. In 1970, the last year for which figures are available, the accident rate per million kilometres travelled was 0.7 in the US, 1.3 in the UK and France and 2.3 in Israel. Only the drivers of Japan and Belgium were more lethal.

In an attempt to cut down on the carnage, the Israeli police have decided to adopt a plan proposed by plasma physicist turned traffic expert

Gerry Ben-David, who aims at re-educating the entire population of Israeli drivers. It is based on putting drivers under close observation and swiftly making them aware of their mistakes. First tried out on a very limited basis, it will now encompass 10% of Israeli highways.

Placed at strategic points along these roads will be specially trained teams, each member of which will concentrate on certain traffic violations. Every violation will be recorded, as will the licence number of the car concerned (though no attempt will be made to stop the vehicle). The violation data will be transferred for computer processing using standard techniques, and the computer programme will be then control all subsequent communication with the driver. Computerisation makes it possible to ensure that within a matter of days the person (or company) in whose name the car is registered will receive a letter from the traffic police giving full details of the offence and pointing out the dangers involved in such behaviour. If the owner himself was not driving his vehicle at the time, he will be asked to identify the actual driver so that he can be contacted.

During the initial, small-scale trial of the Ben-David scheme, observers witnessed an extraordinary number of traffic violations. One four-man team, for example, reported 6,086 offences in one month along just one section of the road, the most common violations being close driving, unnecessary driving in the lane reserved for passing, and speeding. Motorists "caught" up to three times were sent letters, while those guilty of more traffic violations were asked to come for a talk with the police or perhaps even forced to undergo a new driving test before being allowed to continue on the road.

While Dr Ben-David cannot yet prove that his scheme actually prevents traffic accidents, he notes that a follow-up carried out during the preliminary trial of his system showed that the observed violation rate dropped more than 50% for drivers who received a single letter and 80% for those who received three letters. When observation teams are operating over large stretches of Israeli highways, he feels certain that there will be a significant drop in the countrywide accident rate.

Accident prevention in Israel involves special problems, notably the fact that 75% of the drivers have been on the road for less than 10 years. At the same time, drunkenness, a major cause of accidents in other Western countries, is practically un-

known. In spite of the differences, if Dr Ben-David's system works in Israel it may also work elsewhere.

● After a hard day of driving, exhausted Israelis slump into their arm-chairs and turn on the TV, anxious for entertainment rather than education. It may be for this reason that Israeli TV allocates so little time for science, particularly locally produced programmes.

This month, however, viewers were introduced to an excellent new TV series on archaeology which gives them both science and entertainment, the latter stemming from the fact that the man who explains the archaeology is a master entertainer. Hebrew University Professor Yigael Yadin is no ordinary archaeologist: his descriptions of an excavation have the artistry of a well-crafted murder mystery, and an audience of laymen is kept breathlessly alert awaiting the discovery of an artifact which will reveal the true identity of an ancient civilisation or the cataclysmic event which brought about its destruction.

Yadin's archaeology programme features a "Find of the Month" and other recent discoveries as well as new interpretations of previously unearthed objects. One of the latter, which Yadin showed viewers on his last telecast, was a 6,000-year-old vessel with "beaks" which, until recently, had been called a "bird-like utensil" for want of a better term. Now, he explained, archaeologists have discovered its real purpose by taking a close look at the "food technology" of contemporary bedouin. As a film sequence convincingly demonstrated, the bedouin use a very similar container to make cheese and other processed milk products, which means, almost certainly, that the "bird-like utensil" was an ancient churn.

Another regular feature of the series, "Archaeology Around the Corner", highlights the fact that almost every Israeli town and village has one or more sites of interest. Yadin recently zeroed in on a site in West Jerusalem, where a partially exposed column, lying flat, is clearly visible. A camera team was stationed near the object and then passers-by were asked what it was. Only after several rather comical replies did an interviewee correctly state that it was a column intended for use in the construction of the Second (Herodian) Temple which for some reason had been left lying several miles from the Temple site.

Nechemia Meyers
Rehovot

NUCLEAR BRIEF

US export moratorium ?

Proliferation of the international trade in nuclear technology is "very rapidly becoming a major political issue in the United States", Mr Llewellyn King, Editor of *Weekly Energy Report*, told the *Financial Times* conference on nuclear power last week. Hearings set for July 20 on the subject would bring it into the front line, and if proliferation became an issue in the coming elections, he predicted, there would be a "de facto moratorium on the export of nuclear technology", a change which would affect the world nuclear market. The issue was a "lively" one in Congress, and the President was "very concerned".

Reprocessing prospects

The prospects for the nuclear fuel reprocessing deal that British Nuclear Fuels Ltd (BNFL) is negotiating with Japan look "very good indeed", and there has been "very good progress" in negotiations with European countries for similar business, according to Mr Con Allday, managing director of the company. The deal, which involves BNFL's reprocessing facility at Windscale, attracted a good deal of publicity earlier this year, since when the French firm Cogema has been introduced on one side and changes have occurred in the team on the Japanese side. An Anglo-French mission recently visited Japan for talks.

Uranium fix

Canada is resisting the efforts of the US Justice Department, which has served subpoenas on two of the country's three major uranium producers, to investigate alleged attempts at price fixing. The chairman of one of the companies is also president of the Uranium Institute, the trade association representing the industry's producers and consumers which recently held its first annual general meeting in London. It has not been served with a subpoena, and stresses that its activity does not and would not include price fixing. Several companies in the United States have also been served with subpoenas.

THE pressures on Britain's countryside increase, and so do the efforts of those concerned with wildlife conservation. Since the 1939-45 War, 146 National Nature Reserves covering nearly 120 thousand hectares have been designated, and voluntary organisations such as the Royal Society for the Protection of Birds, the National Trust, and the County Naturalists Trusts have obtained control of only slightly less ground. In these days of world shortages, and the need to produce more food in Britain to reduce imports and the balance of payments deficit, there is concern as to whether it is right to set aside so much land for the benefit of the minority of the population interested in our native animals and plants.

At a meeting recently organised by the Royal Society on the scientific aspects of nature conservation in Britain, two main topics were discussed. The first was the value of conservation as such; the second concerned the contribution of science, particularly ecology, to conservation and the management of nature reserves and other protected areas.

The conservation of the land, of water and of natural resources is clearly desirable, and wildlife may be looked upon as just another "resource". The difficulty is to give this a value, particularly a cash value. Many of the arguments about the economic importance of wildlife are unconvincing to the layman, and may prove counterproductive. It is true that several wild birds and plants may act as inexpensive and delicate indicators of pollution, but the important species are not confined to nature reserves. Wild plants have proved valuable in work in producing new crops, but it is difficult to argue convincingly that the gene pool of the Spring Gentian in upper Teesdale has

a contribution to make to food production.

It is safer to base one's support for wildlife conservation on scientific, educational, cultural, aesthetic, recreational or even sentimental grounds. The scientific argument

Nature conservation



KENNETH MELLANBY

would perhaps appear to be the strongest, but even here the conservationist must not appear to claim too much. Nature reserves provide essential facilities for ecological research, and so make an important contribution to that science. However, we may become involved in a circular argument: reserves are provided for research, which yield results which enable us to maintain those reserves so that they continue to provide facilities for more research. To the sceptic this may sound too much like "jobs for the boys". I believe that we conservationists should be honest, and admit that our motivation is not, mainly, economic. We should not be ashamed to admit that our interests

are, at least partly, aesthetic and sentimental, and that we believe that conservation contributes to civilised living.

But if we do not claim that conservation is of economic importance, what do we say to those who complain that we are harming agriculture? Could not the quarter of a million hectares in different types of reserves be better used by our farmers? First, it should be noted that only a tiny fraction of these reserves is grade 1 or grade 2 farm land, suitable for arable cultivation. Secondly, we lose vastly greater areas, often of the best land, to housing, factories, motorways and airports. This loss now amounts to 30 thousand hectares a year; in four years the loss in area exceeds that of the 146 National Nature Reserves. The first priority should be to reduce, and eventually stop, this continuing erosion of our farmland.

In the meantime, conservationists are surely right in trying to acquire as many sites as possible. Modern farming, to be profitable, cannot afford to support wildlife except in marginal areas. We need reserves where wildlife has priority, and some areas should be on good farmland, which supports larger numbers of more species than the barren moors generally left to the conservationist. We certainly need to produce more food, but first let us cut out waste. Nearly a quarter of the food bought in our supermarkets ends up in our dustbins, and intensive livestock rearing wastes over ninety per cent of the rations fed. So long as these processes continue, conservationists need have few qualms when they designate new reserves, particularly as this land, unlike that used by industry, could easily be brought into fruitful cultivation in an emergency.

correspondence

Electrostatic energy storage

SIR,—With shortages of oil and petrol clearly facing us, it is time to consider all other ways of storing energy effectively. Although leading possibilities for automotive use include advanced electric batteries and specially designed flywheels, the search for materials (particularly polymers) with high dielectric strength and static dielectric constant must not be overlooked; it might lead to economically attractive electrostatic storage systems.

The efficiency in converting the stored electric energy to mechanical energy to turn wheels can confidently be expected to be very high—close to 90%. This means that substantially less energy (about 1/7) need be stored in a vehicle for range, speed and acceleration on a par with a petrol-burning system. There is no exhaust to pollute the air and even thermal pollution by wasted heat is reduced by a factor of 9 or so at the vehicle. Recharging can be done more rapidly and efficiently than conventional battery charging: a few minutes should suffice at a service station equipped with suitable rectifying equipment and electricity supply lines of industrial capacity. Alternatively, the domestic electricity supply is generally adequate for overnight charging. With programmed parallel-series switching the variation in operating voltage can be kept modest and the extraction of stored energy can be made nearly complete.

The stored energy density in a sheet of dielectric can be expressed as

$$D = 0.4427 \times 10^6 K a^2$$

with D in kJ m^{-3} and a in $\text{V } \text{\AA}^{-1}$; K is the low frequency dielectric constant. Many commonly used capacitor dielectrics have dielectric constants below 10, corresponding to strong ionic or more

often to non-polar interatomic bonds. A class of metal titanate ceramics, however, has dielectric constants up to 12,000. But the dielectric strength is the more critical parameter and is sharply reduced by various impurities. In paper-thin sheets, appropriate to capacitor construction, high purity can be achieved in mica ($a \approx 0.022$) and organic polymers ($a \lesssim 0.028$).

Applying the energy storage formula for mica with a dielectric constant of ~ 5 to 7, we might expect a storage capacity of 1,000–1,500 kJ m^{-3} of mica. Commercial energy storage capacitors are listed up to 600 kJ m^{-3} , based on a waxed paper dielectric, so this level of energy density is close to commercial practice. Recognising that there will be some additional volume required for the thin electrodes and an outer casing, a comparable organic polymer capacitor should have approximately the density of water, and thus an energy density of 1–2 kJ kg^{-1} .

If we take the lead-acid storage battery, at 44 kJ kg^{-1} , as an aiming point, we would need to develop a material with the dielectric strength of the best plastic, incorporating enough polarisable sites to raise the dielectric constant above 130. This is a reasonable aim in economic terms as the lead-acid battery is already marginally adequate for electric cars and trucks.

A further discussion of promising materials and ideas for study has been prepared (ORNL Report TM-29, June 1976).

Yours faithfully,

R. L. MACKLIN

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Electrostatic energy discharge from humans

SIR,—The amount of electrostatic energy which can be stored in the capacitance represented by the human body does not appear to have been measured and reported in the literature. Besides being of phenomenological interest, this measurement could be important when considering the explosion hazard represented by anaesthetics and antiseptics when used in close proximity to electrostatically charged humans, such as in operating theatres and intensive care areas. In this regard, it has been suggested that ether vapour will explode when subjected to an electrostatic energy as small as 200 μjoules in magnitude.

An appreciation for the magnitude of the electrostatic voltage which the human body can be charged to; the capacitance represented by the human form; and the electrostatic energy stored by humans, can be obtained by discharging the body through a known resistance, and displaying the discharge curve on a storage oscilloscope.

The results obtained indicate that the electrostatic energy which the human body can store, and consequently dissipate, is in the order of 6.46 ± 3.26 millijoules. The capacitance of the body is 65.9 ± 12.1 pf, and the electrostatic voltages which the body can be charged to is $13,802 \pm 4,443$ volts. These measurements could be valuable when considering the role that the human body plays in the causing of explosions of volatile chemicals in hospital and industrial environments.

Yours faithfully,

A. E. MARBLE
D. MACVICAR
A. ROBERTS

Halifax, Nova Scotia, Canada



Competition 8

Du Pont's notepaper has "There's a world of things we're doing some-

thing about" imprinted on it, and the slogan for Rockwell International is "Where science gets down to business".

A prize of £10 is offered for the best one-sentence slogan (preferably tongue in cheek) for your (or anyone else's) laboratory, business or university. The closing date for entries is August 31.

Competition 7 requested a modern headline for a scientific achievement,

and there was a large entry, on fairly predictable themes. A. L. Mackay (London) receives honourable mention for "Prominent philosopher in public bath scandal". The £10 prize is shared equally between P. J. Fallon (London), for "Role of Earth probed by Pole", and C. F. Hammer (Wilmington, Delaware), whose "Bell discovers new use for wooden poles—wires to carry conversation over distances" seemed just right for the *New York Times*.

news and views

Interpreting protein electron density maps

from C. C. F. Blake

EVER since the subject began, protein crystallographers have been complaining that they work for a long time on a project without obtaining any significant information and then, literally overnight, they are overwhelmed with information. The source of this surfeit of data is the electron density map of the protein which is calculated in the form of a three dimensional figure-field of electron density values that contains between 50,000 and 500,000 points, depending on the size of the protein and the resolution of the study. Although the figure-field will encompass one protein molecule, much of the data is redundant because it will inevitably include some volumes of liquid containing more or less-ordered water molecules, and parts of other (identical) protein molecules related by symmetry or simple translation to the central molecule.

In view of the vast amount of information present in the figure-field, total interpretation is not normally attempted, attention being naturally concentrated on the folding of the polypeptide chain and the location of side chains. This operation has always been carried out in the past by converting the figure-field into a stack of transparent sheets contoured in levels of equal electron density, and interpreted in terms of a physical molecular model. This is, however, by no means straightforward, especially if an accurate model is required, because the ideal of building the model inside the electron density is a physical impossibility. After various laborious *ad hoc* methods had been used on the first proteins, a standard piece of apparatus—the optical comparator—was proposed by Richards (*J. molec. Biol.*, **37**, 255; 1968), using the ingenious principle of optical superposition of the model and the contoured electron density map. Although it is relatively easy to use, the comparator has certain disadvantages: it is large and uses up valuable laboratory space; with larger proteins it becomes physically difficult to build the model, and some users have had difficulty with depth perception. Another problem associated with all physical models, however they are built, is the need to measure the spatial coordinates of all non-

hydrogen atoms, a laborious process requiring some thousands to tens of thousands of accurate measurements. These coordinates are then returned to the computer, either for drawings to illustrate the structure, or for some kind of refinement process for improving their accuracy.

Since the current system requires very hard work to obtain a somewhat inaccurate model it seems desirable to seek an improved system, which clearly has to be computer based. The most obvious improvement is to use a computer display system as an electronic Richards' comparator, as proposed by Katz and Levinthal (*A. Rev. Biophys. Bioengng.*, **1**, 465–504; 1972). This requires expensive hardware and allows only a limited section of the map to be displayed for fitting at any one time. An alternative is to use the basic computer to attempt the appropriate pattern recognition on the figure-field in an automated or semi-automated procedure. This approach, whose development has been reported by Greer in a series of papers (Greer, *J. molec. Biol.*, **82**, 279; 1974; **98**, 649; 1975; **100**, 427; 1976) has now reached the point where it has been used successfully to trace the main chain and produce a preliminary set of atomic coordinates of a Bence-Jones protein (Greer, *J. molec. Biol.*, **104**, 371; 1976).

The basic aim of Greer's approach is to reduce the electron density to a series of thin connected lines that trace the path of the polypeptide chain. Although the path of the polypeptide chain will be marked by a continuous string of above average density, in any real protein map it is by no means uniquely defined. Variation in thermal vibrations and possibly even of order will cause the level of the string of density to be quite variable, while disulphide bridges and strong intramolecular or intermolecular hydrogen-bond contacts will result in strong continuous features that do not represent the path of the main chain. These factors result in formidable problems in attempting to recognise and isolate the true main-chain density from these other similar, but spurious features, and Greer has had to develop a procedure that has a number of stages in each of which criteria have

been evolved that successively eliminate density points which do not represent the main chain.

The starting material is the electron density figure-field in a Cartesian coordinate system with a grid spacing of about 1 Å, that completely encloses a single molecule. The first operation is the elimination of all electron density points below a minimum value and "contouring" the remainder at equal intervals. The appropriate values are arrived at by inspection to give maximum main-chain continuity and a minimum of spurious cross connections. Starting with the lowest contour level, the remaining points are successively removed to leave a skeleton of lines that defines the basic connectivity in the map, using a three-dimensional generalisation of a method developed for processing micrographs of chromosome smears. This skeleton is then simplified by pruning easily distinguishable side chains down to the main chain, leaving a flag to mark the position of the α -carbon atoms. At this point the description of the skeleton is transformed into a tree-like structure in which segments are defined as lines that lie between the tips or branch points in the skeleton; such a description restricts ambiguities to the points where segments meet. After this, all segments that represent parts of neighbouring molecules that obstruct into the Cartesian frame are removed to isolate a single molecule. Simple loops in the skeleton are removed next by using simple rules to identify the true main-chain branch. The skeleton that remains represents primarily the main-chain and inter-main-chain bridges, possibly lacking one or two main-chain connections that were removed earlier. The final stage involves putting the skeleton through a process that selects the main chain on the basis of length and the presence of side chains, starting with the longest segment and attempting to add other segments to each end. This is an iterative procedure that includes examining skeletons calculated at lower electron density to locate weak main-chain connections eliminated from the main skeleton. Wisely, manual intervention is allowed for when the programme reaches an

ambiguity that cannot be resolved easily.

Using the procedure on a 3-Å resolution map of the Bence-Jones protein Rhe, Greer has shown that it defined a main-chain line some 433 Å long, produced provisional coordinates for 113 α -carbon atoms, defined the basic β -sheet structure characteristic of Bence-Jones proteins and also found a short segment of α -helix. Even at this stage it is clear that the technique

could be valuable and it seems probable that it can be developed further. Although some crystallographers will see this as a further encroachment of the computer into what they consider to be their proper province, most will note its real value in giving more time (and possibly a better start), for the important task of getting an accurate description of the molecule through which the biological problems can be solved. $\square$

Hydrogenases and efficiency of nitrogen fixation in aerobes

from R. O. D. Dixon

WITH the high energy, and thus financial, cost of nitrogen fertilisers it is now important to rely, where possible, on microbial nitrogen fixation. The fixation of nitrogen by microorganisms occurs with varying efficiency in terms of substrate consumed for each nitrogen fixed. To use microorganisms to the best advantage it is well that we be able to identify factors which lead to greater efficiency of nitrogen fixation and thus to select the most efficient organisms.

It is known that metabolic energy in the form of ATP and reducing power is wasted, by the enzyme nitrogenase reducing protons rather than nitrogen. Evolution of hydrogen occurs with nitrogenase from all nitrogen-fixing microorganisms tested so far *in vitro*, although it has not been possible to test the extent to which this wastage occurs *in vivo* in most organisms because of the complicating presence of hydrogenases. In anaerobic organisms there is a hydrogenase which evolves hydrogen simultaneously with the nitrogenase whereas in aerobes, such as *Azotobacter*, hydrogen is taken up by a hydrogenase.

In experiments in which it was thought that the hydrogenase had been inhibited by carbon monoxide, no hydrogen evolution was detected from *Azotobacter*. It was thus suggested that, in this organism, the nitrogenase, atypically, did not reduce protons. (Postgate, in *The Chemistry and Biochemistry of Nitrogen Fixation*, 161–190, Plenum, New York, 1971). This view, which was not universally accepted, has now been shown to be erroneous by Smith, Hill and Yates (page 209 of this issue of *Nature*) who report that the *Azotobacter* nitrogenase does not in fact differ *in vivo*, from that of the other nitrogen fixers. They confirm that the lack of hydrogen evolution from intact bacteria is due to hydrogen uptake by a hydrogenase.

That the hydrogen, taken up by the

hydrogenase, may be put to some use was shown some time ago by Hyndman *et al.* (*J. Bact.*, **65**, 522–531; 1953). They showed that the hydrogen taken up by *Azotobacter* was oxidised with consequent synthesis of ATP. Similar observations were made more recently with hydrogenase in *Rhizobium* bacteroids (Dixon, *Arch. Mikrobiol.*, **85**, 193–201; 1972). Possession of this hydrogenase enables these aerobic organisms to recoup some of the ATP expended on wasteful evolution of hydrogen by nitrogenase. The suggestion was made earlier (Dixon, in *Nitrogen Fixation by Free-living Microorganisms* (edit. by Stewart, W.D.P.), Cambridge University Press, 1975), and is now reiterated by Smith *et al.*, that this reutilisation of hydrogen with ATP formation is the reason for the greater efficiency which has been found for *Azotobacter* nitrogen fixation over that of anaerobes such as *Clostridium* (Hill, Drozd, and Postgate, *J. appl. Chem. Biotechnol.*, **22**, 541–558; 1972).

Although the presence of hydrogenases in free living organisms has precluded the investigation of the level of wastage by the evolution of hydrogen, the lack of hydrogenase in some root nodule associations has enabled Schubert and Evans (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 1207–1211; 1976) to estimate the degree of efficiency of the fixation of nitrogen in a number of such associations. The more efficient associations, which included all the non-legume root nodules in the study, showed evidence of a hydrogenase which takes up the hydrogen evolved. The wastage of ATP and reducing power by evolution of hydrogen is estimated to vary between 40 and 60% of the total used by nitrogenase in those associations in which hydrogenase was not a complicating factor.

Schubert and Evans calculate that with a wastage of 50%, reutilisation of hydrogen could double the amount of

nitrogen fixed in cases where the energy supply is limiting. It has indeed been demonstrated in a number of cases that the supply of photosynthate from the plant limits nitrogen fixation by root nodules. Although this calculation would seem to be optimistic, if one bears in mind that the estimate of molecules of ATP utilised, per pair of electrons transferred through nitrogenase for hydrogen evolution, is three to four and the P/O ratio obtained from hydrogen uptake is not likely to be better than two, substantial savings can be made. This would be reflected, as Schubert and Evans point out, either in an increase in dry matter yield, where photosynthate is not limiting, or in an increase in nitrogen fixed. Thus selection of a strain of *Rhizobium* which possesses this hydrogenase may pay dividends in the yields of some legume crops.

Much work has been done and is being pursued on the legume–root nodule association in order to understand the infection process and nodule development, with the long term aim of transferring nodulating capability to other agricultural crops, whereas little attention has been paid to non-legume associations. But the greater efficiency of nitrogen fixation of non-legume associations shown in the data of Schubert and Evans suggests that it may be well to give them more attention than in the past. $\square$

Irregular associations

from E. G. Richards

FOR nearly a decade it has been known that complementary trinucleotides do not associate detectably. So, we may ask, how does the anticodon on the tRNA bind with such specificity to messenger triplets on the ribosome? Part, at least, of the answer must lie in the tRNA, for trinucleotides complementary to anticodons of tRNA associate with the latter and, more dramatically, as Eisinger showed some years ago, pairs of tRNA molecules with complementary anticodons bind together with an association constant as high as 10^6 M^{-1} .

The purist may wonder what relevance the participation of tRNA molecules in these unnatural acts may have to tRNA–messenger interaction but Grosjean, Söll and Crothers have come up with some interesting suggestions (*J. molec. Biol.*, **102**, 499; 1976).

When such pairs of tRNA molecules interact, they do so with a small (0.1% of the total) absorbance change which

Regulatory genes in fungi

from a Correspondent

THE paper by Arst in this issue of *Nature* (page 231) marks another step in our understanding of regulation of gene action in the mould *Aspergillus nidulans*. In 1970, Hynes and Pateman (*Molec. gen. Genet.*, **108**, 97–106, 107–116) reported mutants which identified a gene whose product seemed to act as a positive regulator of the structural gene for amidase (*amdS*). Now Arst reports that this regulatory gene—now called *intA* for integrator—also regulates expression of two genes concerned with ω -amino acid metabolism. These latter two genes, *gatA* and *gabA*, code, respectively, for an inducible transaminase acting on β -alanine, γ -aminobutyrate or δ -aminovalerate and a permease for the same ω -amino acids. The three genes under control are all unlinked to *intA* and to each other. It seems that various mutations in *intA* can affect the control of *amdS*, *gatA* and *gabA* differentially, and can also be associated with changes in the relative responses to γ -aminobutyrate and β -alanine as effectors. The conclusion is that the *intA* product interacts with each of the three genes (or with controlling regions associated with them) and also with various small molecular effectors, and that these specificities can be altered by mutation. Arst draws attention to the similarity between *intA* and the type of integrator gene proposed by Britten and Davidson in 1969 (*Science*, **165**, 349–357) as part of an influential general model for regulation of gene action in eukaryotes.

Arst's report, together with earlier work, shows that sets of unlinked genes

can be simultaneously subject to more than one system of integrative regulation. Arst and Cove and their colleagues (for example, *Molec. gen. Genet.*, **126**, 111–141; 1973) had already described in *Aspergillus* a regulator gene, called *areA*, which is concerned with nitrogen catabolite repression, and which regulates the action of several other genes with functions in the generation of ammonium ion. A type of mutant allele, called *areA^r*, represses these other genes (including the amidase gene) even in the absence of ammonia. "Constitutive" alleles of *intA* can nullify the repressing effect of *areA^r*, the positive integrative control in this case over-riding the negative. In addition, one can have positive gene-specific regulation, acting in parallel with the "integrator" regulation. Thus, another regulatory gene, *amdA*, is found by Arst to act positively on *amdS* (more or less additively with *intA*) with no effect on *gabA* and *gatA*.

The regulation of nitrogen metabolism in *Aspergillus*, thanks mainly to the work of J. A. Pateman in Glasgow, Arst (with D. J. Cove and C. Scazzocchio) in Cambridge, M. J. Hynes in Australia and P. Weglenski in Warsaw, is becoming an extremely complex story—excessively so, perhaps, in the opinion of some. But the complexity is there in the eukaryotic cell. Attempts to unravel the network of gene interactions are bound to produce something of a tangle at the outset, but perhaps some general features are beginning to emerge which will eventually fall into place in a higher-order pattern.

specific cleavage of the first 16 nucleotides from the 5' end of the tRNA^{Phe} did not alter the interaction. On the other hand, fragments obtained from both these species such that anticodon helical arms could not form, still associated but with a constant reduced by about two orders of magnitude—leaving an enhancement of four orders still to be accounted for.

The remaining factors were deemed to reside in the "dangling ends" or the residual four bases in the anticodon loop that are not involved in the anticodon itself. Grosjean *et al.* point out that the sixth base in the anticodon loop (that is the one at the 3' end of the anticodon) is frequently modified. They go on to show that the association constant varies for species with similar anticodons but differs in the nature of the sixth base, and, in particular, that excision of the Y base from this position in tRNA^{Phe} leads to a substantial reduction in the enthalpy of association.

Grosjean *et al.* tie all these observations together by postulating that the enhanced association constant and the increased enthalpy of association originates from the stacking of the two anticodon arm helices on either side of the helical region formed by the anticodon-anticodon interaction, with the 3' dangling ends stacked up as jam in this three layer cake. I choose this analogy carefully since jam is well known to be sticky.

We are finally left with the thought that something similar happens in the codon-anticodon interaction. Maybe the ribosome holds the relevant portion of the messenger in a configuration similar to that in the tRNA and allows the interaction to be similarly stabilised by stacking.

These results also imply that the anticodon helical arm exists in both the native and denatured forms of *E. coli* tRNA^{Glu}. Further evidence of the similarities and differences between these two forms of tRNA comes from a paper by Jones, Kearns and Muench (*J. molec. Biol.*, **103**, 747: 1976) who worked with *E. coli* tRNA^{Trp}. They measured the low field nuclear magnetic resonance (NMR) spectrum of the native form of this species and assigned to their satisfaction all parts of the 11.5–14.5 p.p.m. region to one or other structural feature expected of the clover leaf model in its surmised tertiary structure. There were thus 19 base pairs, a protected U or G and two tertiary base pairs including A₁₄-s²U₈. The agreement between their experimental spectrum and that computed from these assignments was very reasonable. They then repeated the process for the denatured form and considered in detail the difference NMR spectrum between the two. They

Grosjean *et al.* were able to monitor in temperature jump relaxation experiments. This absorbance change shows the right spectral distribution to be expected from the base pairs presumed to be formed, and is not manifest when the two anticodons are not complementary. Moreover, the three complementary bases must be centred in the middle of each seven-membered anticodon loop for the reaction to be appreciably manifest. Thus, for interaction, the anticodons *per se* must be complementary, and complementarity between other bits of the anticodon loops does not help.

Returning to the temperature jump results, the authors found that the rate constant for the association reaction was close to that expected for the association of complementary trinucleotides, and, furthermore, virtually temperature independent. The surprise comes in the rate constant for the back dissociation reaction which was six orders of magnitude slower than ex-

pected. It follows that the equilibrium constant is 10⁶ times higher than expected.

More detailed analysis showed that the entropy of interaction was not very different from that expected for trinucleotide interaction, but the enthalpy change was about –25 kcalories, which is about 10 kcalories larger than expected. This difference of 10 kcalories is sufficient to account for the factor at 10⁶ and is at the kernel of the enhanced anticodon-anticodon interaction. This in itself suggested that stacking interactions were involved.

Pushing the investigation further, Grosjean *et al.* studied the interaction of *Escherichia coli* tRNA^{Glu} with yeast tRNA^{Phe} in the presence of EDTA, in which conditions the former species is in the denatured conformational state. The association was still observed, suggesting that the structural features which distinguish the two conformational states are not important to the association. Similarly the removal by

succeeded again in demonstrating a reasonable agreement between calculated and experimental spectra. The final result is a model for the denatured form in which stem, TUC arm and anticodon arm remain intact but the DiHU arm is undone and the anticodon arm extended by some of the original DiHU arm bases pairing with bases in the extra arm. This extension of four base-pairs involves one bulged out G. This model they argue is also consistent with the pattern of methoxylation of both the native and denatured forms. In an argument too complex to repeat here they also consider and reject other possible pairing schemes for the denatured form.

Besides being consistent with their own data the model for the denatured form has several attractions. The small number of base pairs that needs to be undone for either form to revert to the other is consistent with the ease of this interconversion. Furthermore, if it is assumed that *E. coli* tRNA^{Tyr} has a tertiary structure similar to that of yeast tRNA^{Phe} as vouchsafed by X-ray diffraction analysis, then this interconversion can be readily envisaged to occur with but minimal disruption of the tertiary structure.

If all this is accepted we can begin to point at those parts of the structure that are involved in the biological activities that are knocked out when the molecule is denatured. □

Meteoroid swarms colliding with the Moon

from David W. Hughes

METEORIC dust particles are continually striking the Moon with velocities between 2.4 and 74 km s⁻¹. On impact they lose their considerable kinetic energy and excavate a crater thus producing a large quantity of hot ejector most of which subsequently falls back to the lunar surface but some of which exceeds the escape velocity and moves off into space. A lunar seismic disturbance is also produced. The latter can be detected by the passive seismic network deployed by the Apollo astronauts. This consists of four stations arranged around an approximately equilateral triangle with sides about 1,100 km long. The Apollo 15 seismometer makes the northern apex at Hadley-Appenines, Apollo 16 is in the east in the crater Descartes, and the west apex has Apollo 12 in Oceanus Procellarum and, 180 km away, Apollo 14 at Fra Mauro. Each station has three long period (>1 s) seismometers, sensitive to motion along three orthogonal directions, and a fourth short period (<1 s) seismometer sensitive to vertical motion. Each can detect a ground movement of about 0.05 nm.

Fortunately lunar scientists think that the seismic signals produced by meteoroid impacts can be distinguished from those caused by moonquakes. Impact events have sharper first signal rise times, indistinct shear wave arrivals and lower frequencies in the wave train. This is due to the fact that the impact waves must traverse the surface scattering zone twice between the impact point and the seismometer. Moonquakes, caused by tectonic strains within the lunar interior cross the zone only once so the energy in their seismic wave is less diffuse.

During the 924 days between January 1, 1973 and July 13, 1975 the seismic network detected 815 signals

interpreted as being caused by meteoroid impacts. These have been statistically analysed by Duennebie, Nakamura, Latham and Dorman (Geophysics Laboratory, University of Texas) in their recent paper in *Science* (192, 1000; 1976). If an event is detected at only one seismometer it is regarded as "small". The signal amplitude is probably proportional to $E^{1/2}/r$ where E is the energy of the meteoroid and r is the distance between the sensor and the impact point, so most of the small events are caused by impacting particles with masses between 50 and 10³ g, the larger events, detected at two or more stations, being caused by 5 × 10³ to 5 × 10⁴ g meteoroids. The authors find that a plot of impact rate as a function of time shows several sharp peaks in activity. Also, the frequency of intervals where there are no impacts is significantly more than expected—expected, that is, if the meteoroids are scattered randomly in space and the resultant impact rates follow a binomial distribution. Duennebie *et al.* conclude that some of the objects in the 50 to 50,000 g size range are not distributed randomly in space but occur in swarms. One of the more impressive swarms detected by the network was estimated to be about 2 × 10⁷ km across and have a total mass between 10¹³ and 10¹⁴ g.

Could these peaks in the rate curve be caused by the Moon passing through a stream of particles produced by a decaying or decayed comet? Meteor showers caused by just such a process are common on Earth. The problem is that only about 10% of the mass in a meteor stream is made up of particles with individual masses over 50 g (according to Hughes, *Space Res.* XV, 565; 1975). A considerably smaller percentage of the sporadic Solar

System dust cloud is made up of these large particles, however, so the meteor streams should stand out prominently above the background. Now 50 and 50,000 g particles impacting with the Earth's upper atmosphere at around 40 km s⁻¹ produce visual meteors of magnitude about -4 and -12 respectively and an increase in flux of these very bright meteors and fireballs has been noticed during meteor showers. Meteor streams are always detected from Earth at the same solar longitude (about the same time each year) and Duennebie *et al.* note that the lunar events show no yearly pattern. The fact that the Moon oscillates by 3.8 × 10⁵ km about the Earth's orbit does not help to get over this problem as meteor streams such as the Perseids and Geminids are 2.0 × 10⁶ and 1.0 × 10⁶ km wide. Both these streams are rich in fireballs.

It was also noticed that the mass distribution of the incident particles changed during the high rate events, the percentage of smaller objects being larger in the swarms than during the normal sporadic influx. Unfortunately this is exactly the opposite to what is observed with visual meteor showers, where the percentage of large particles increases.

Two of the maxima occurred on consecutive months, near new Moon, whereas the June, 1975 maxima occurred at full Moon. This contrasts with the timing of the moonquakes observed at the Apollo 17 site (Cooper and Kovach, *Proc. Lunar Sci. Conf.* 6th, 2863; 1975) which appear to be closely correlated with the 29-d lunar month and the 300 K temperature variation of the lunar surface layer that occurs between mid-day and mid-night.

So these data reveal something of a mystery. Large moonquake rates result, according to the authors, from the impact with the Moon of swarms of particles with masses greater than 50 g. Should similar swarms be seen on Earth? The answer is no, because even the peak lunar activity only corresponds to an infall of one large meteoroid every 3 days over an area 100 km in radius. The converse though is puzzling. The annual meteor streams seen on Earth contain a significant flux of particles in the mass range supposedly detected on the Moon. This flux also exceeds the sporadic (non-shower) flux by nearly an order of magnitude. So why haven't these been observed in lunar seismic data? Using the influx rates for the large meteoroids which produce fireballs in the Earth's atmosphere, obtained from the American Prairie Network of all-sky cameras (McCrosky and Ceplecha in *Meteorite Res.*, 600 (edit. by Milman, P.,) D. Reidel, Dordrecht, 1969), about

250 particles with masses greater than 50 g should hit the Moon each day. Even going to the upper limit (50,000 g), of Duennebier *et al.*'s mass range the rate is about 10 per day. This compares with the maximum rate of 12 events per day observed by the seismic network. If meteoroid impacts are being detected the major meteor showers should stand out clearly above the sporadic background. The fact that Duennebier *et al.* are not seeing these showers introduces the possibility that they are seeing something else. □

Metals in proteins

from S. D. Dover

THE environment of metals in metalloproteins is of growing interest with the advent of synchrotron radiation. As distinct from crystallographic studies, which reveal the whole molecule, X-ray absorption measurements in the neighbourhood of the absorption edge of a metal are indicative of the local environment.

The use of synchrotron radiation as a tool for studying that environment is probably at the beginning of its exponential growth phase. In this application a tunable source of X radiation is required so that the spectrum can be observed. Conventional tubes are designed to emit most of their X-ray energy in the characteristic radiation line. The white radiation background, for a similar wavelength spread, has a much lower intensity. This means that the power available from the synchrotron over a whole range of wavelengths is several orders of magnitude greater than from laboratory sources.

This factor makes it possible to measure in an acceptable time the X-ray absorption spectra of systems where the absorber is relatively dilute (as in metalloproteins). Results from the Stanford Synchrotron Radiation Project (SPEAR) have been reported (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2340; 1975) where gains in speed of 10^4 – 10^5 over X-ray tubes have been obtained. For example, the spectrum for haemoglobin over a 400 eV range was measured at 1 eV steps in only 20 h. This gave an acceptably low noise compared with the modulation of the X-ray absorption.

It is the modulation of the absorption spectrum that is of interest. The spectrum of a free metal consists of a low absorption region below the absorption edge where the incident photon is insufficiently energetic to excite the K electron. With increasing energy of X rays, the absorption edge is reached as the K electron can be

excited to empty bound states. Beyond the edge itself the photon energy is sufficient to cause the emission of a photoelectron. The absorption tends to decrease monotonically from the absorption edge.

In contrast the spectrum of a bound metal in this region (the region of the extended X-ray absorption fine structure, EXAFS) is affected by the photoelectron being scattered back to the metal emitter by neighbouring atoms, inhibiting absorption. Instead of decreasing monotonically, the spectrum is modulated as the wavelength of the photoelectron varies with the wavelength of the X-ray photon. It is as if the photoelectron is acting as a diffraction probe for the environment of the metal. The back-scattering from surrounding atoms will have the appearance of interference fringes according to their distances from the metal and the photoelectron wavelength. Theoretical analysis of the fine structure of the spectrum has been successful in terms of the atomic environment for metals themselves (*Adv. X-ray Anal.*, **13**, 248; 1970) and the iron-sulphur system in rubredoxin (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 4003; 1975). Work is continuing on haemoglobin and metalloporphyrins (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2340; 1975) where the results from the porphyrins can be used in the interpretation of the environment of the haem iron.

The absorption edge itself is being studied in model compounds (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 1384; 1976) where the fine structure and position is affected by the available bound electronic states. These and other model compound studies together with those of metalloproteins provide the possibility of examining the electronic situation of metals in proteins, and the two regions of the X-ray absorption spectrum together hold the promise of revealing the whole local environment. □

Fission of medium weight nuclei

from P. E. Hodgson

THE fission of heavy nuclei such as uranium and plutonium is a well known phenomenon of practical importance. In special circumstances lighter nuclei can also undergo fission, and evidence for the fission of nuclei of mass around 80 has recently been found by Braun-Munzinger and colleagues (*Phys. Rev. Lett.*, **36**, 849; 1976).

The experiment giving evidence of such fission is the interaction of 140 MeV ^{32}S

ions with ^{50}Ti . This type of interaction has been studied for many years in order to determine the characteristics of heavy ion reactions, but the particular feature of the new experiment is an array of electronic counters that facilitates determination of the masses and energies of two of the products of the reaction. For

The cytonet protein

IN his report on the recent EMBO symposium, Bretscher (*Nature*, **261**, 336; 1976) refers to the protein which Pearse (*J. molec. Biol.*, **97**, 93; 1975; *Proc. natn. Acad. Sci. U.S.A.*, **73**, 1255; 1976) has shown to be associated with vesicle "coats". In separate publications on this same material in the biochemical and neuroanatomical literature, an important correlation appears to have gone unnoticed. Gray (*J. Neurocytol.*, **1**, 363; 1972; *Brain Res.*, **62**, 329; 1973) examined coated vesicles in presynaptic terminals in the central nervous system and concluded that the coat material is structurally indistinguishable from a fibrous network filling the inter-vesicular cytoplasm. He also noticed that coated vesicles are surrounded by a clear space which he interprets as a retraction zone caused by fracture of an extensive cytoplasmic network, part of which has artefactually collapsed onto the vesicle forming the coat. The coat then appears to be a distorted fragment of a diffuse yet discrete cytoplasmic organelle composed of a fibrous reticulum which Gray called the 'cytonet'.

Gray (*J. Neurocytol.*, **4**, 315; 1975) later repudiated not only vesicular coats but the entire cytonet structure as artefactual, thinking it to be composed of denatured protein complexes. However, Pearse's discovery that vesicular coats contain essentially one protein supports an interpretation of the cytonet as a distinct supramolecular structure. Since it pervades the cytoplasm of many cell types it seems likely to play some role in the spatial organisation of cytoplasmic components, particularly of organelles such as synaptic vesicles. It thus seems important that the protein which coats isolated vesicles should be considered in its broader association with the cytonet, rather than in the limited context of "coated vesicles" which subcellular fraction studies imply. Pearse's proposed name for this protein (clathrin or "coat substance") is a misnomer. If the molecule must have a name 'cytonexin' would be better.

A. J. MATUS

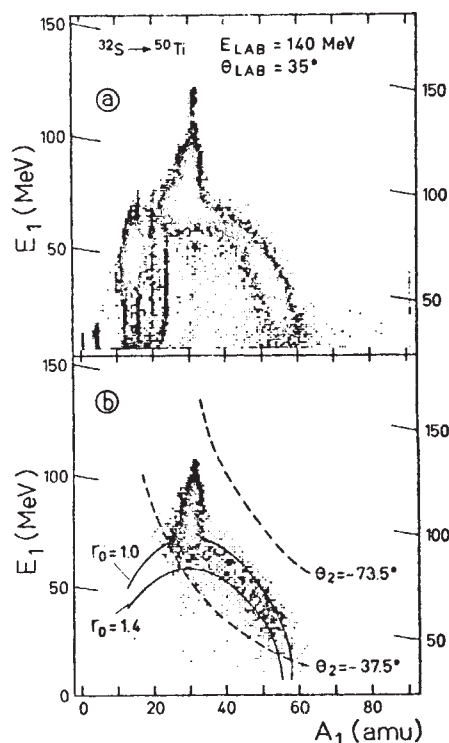


Fig. 1 Plot of the energy and mass number of particles emitted from the interaction of 140 MeV ^{32}S with ^{50}Ti . The top diagram shows all particles and the lower one only those emitted in coincidence with a second particle entering an ionisation chamber. The solid lines are calculated for the electrostatic repulsion of two spheres and the dashed lines are the kinematic constraints due to the detector geometry.

each interaction it is possible to measure the energy (E_1) and mass (A_1) of one of the reaction products by a time-of-flight telescope set at 35° to the incident beam and then the energy and energy loss in an ionisation chamber of a second particle emitted on the opposite side of the beam at the same time as the first, together with its angles of emission in and normal to the scattering plane.

Some of the results are shown in the figure in which each point represents a particle of energy E_1 and mass A_1 detected by the time-of-flight telescope. The top part shows all particles and the lower part only those emitted in coincidence with a second particle entering the ionisation chamber.

In these plots, the vertical lines around $A = 32$ correspond to the incident ^{32}S ions that have undergone peripheral elastic or quasielastic interactions with the loss or gain of a few nucleons. These interactions have been extensively studied and are now quite well understood. The broad band of points extending from $A \approx 32$ to $A \approx 50$ corresponds mostly to particles coming from the fission of the compound nucleus with $A \gtrsim 82$ into two fragments of similar mass.

This identification is made by calculating the kinetic energies for the electrostatic repulsion of two charged spheres separated by $\{r_0(A_1^{1/3} + A_2^{1/3}) + 2\}$ fm for

$r_0 = 1.0$ and 1.4 ; with the lighter fragments following the stability valley. The results of this calculation are shown by the solid lines in the figure, and they follow the experimental distribution very well.

Confirmation of this comes from the identification and kinematic correlations of the coincident particles. The ionisation chamber measurements of the second particle give its charge Z_2 , and assuming its mass is $A_2 = 2Z_2 + 1$, the total mass of the fissioning nucleus is $A_1 + A_2$. For all values of Z_2 between 12 and 16 the average value of $A_1 + A_2$ is between 78.2 and 79.2 compared with the known total $A = 82$. This indicates that on the average about three nucleons are lost in the reaction. The mass distribution of the fission products is uniform from $A \approx 35$ to $A \approx 55$.

Additional studies of the energy distributions of the secondary particles also confirmed that fission had taken place. The correlation between A_1 and the angle of emission of the second particle showed that several nucleons are evaporated during the reaction, but it was not possible to distinguish between nucleons emitted before or after the fission process.

This experiment gives the first definite evidence for fission in this mass region and provides a way of studying the fission dynamics of highly excited nuclei with large angular momenta. □

Suspicious husbands

by John Krebs

ON evolutionary grounds we would expect husbands to be suspicious. If a male is about to spend considerable time and effort in helping his mate to rear young he ought (in evolutionary terms) to check carefully that the young carry his genes. Male lions and langurs may even go to the extreme of killing off all the extant babies when they first take over a new harem: this ensures that the females will only rear children of the new group leader. The female herself, being equally related to her offspring regardless of the father, is not necessarily averse to infidelity.

While one can easily amuse oneself by developing such arguments as the logical outcome of the rules of natural selection (and perhaps even speculate on human analogies) it is not often that one comes across an experimental test. However, Erickson and Zenone (*Science*, **192**, 1353–54; 1976) have recently shown that male ring doves behave exactly as predicted by the theory.

The male ring dove shares with his mate the parental duties of nest building, incubation, and feeding the young, and so should be suspicious of a female

which might have been inseminated by another male. The basis of Erickson and Zenone's experiment was the well established observation that male courtship displays have an important effect in stimulating ovarian activity. After a certain amount of courtship from a male, the female ring dove secretes ovarian steroids which cause her to start the so-called nest soliciting display, indicating her readiness to build a nest. Erickson and Zenone tested the reaction of 35 males to two groups of females: one that had been pre-exposed to courting males and brought into nesting condition, and one that had been kept in isolation. The first group of females immediately started nest soliciting when brought into contact with the test males, while the others did not. The reaction of the males was clear: they showed more aggression and less courtship towards the 'sexed up' females. Thus, as the theory predicts, males were suspicious of females that gave away the fact that they had been consorting with other males, and could have been fertilised by them. □

Haemopoiesis discussed

from J. J. T. Owen

A symposium on Haemopoiesis in Vertebrate Embryos was held at the Institut d'Embryologie, Nogent-sur-Marne, France, on June 14–16. It was sponsored by the Delegation Generale à la Recherche Scientifique et Technique, Centre National de la Recherche Scientifique and the International Society of Developmental Biologists.

MANY meetings these days seem to concentrate on highly specialist topics; perhaps it may be said that we learn more and more about less and less. This meeting was different and the organising committee should be congratulated on getting together scientists from various disciplines including embryology, immunology and haematology.

E. Cooper (Los Angeles) opened the meeting with an account of the evolution of blood cells and pointed out that the most primitive blood cell, a protohaemocyte, was involved in phagocytosis and nutrition. A lively debate followed about the origins of haemopoietic stem cells during ontogeny—an important topic that has generated a good deal of heat since the days of Maximow. In particular, the origins of thymus lymphocytes attracted special

interest—do they arise from the thymic rudiment itself or from blood-borne stem cells? E. P. Volpe (Tulane University) and N. Cohen (Basel) argued that experiments in which tissue primordia were transplanted between frog embryos of different ploidy states, proved that thymus lymphocytes are derived by differentiation of cells intrinsic to the thymic rudiment itself. Furthermore, Volpe suggested that a similar situation may exist in birds and mammals. But the elegant transplantation experiments of N. Le Douarin and her colleagues (Nogent-sur-Marne), using the structural differences between quail and chick nuclei as markers, demonstrate clearly that lymphocytes of the thymus and the bursa of Fabricius are derived from blood-borne stem cells. In addition, P. Deparis and A. Jaylet (Toulouse) and J. Charlemagne (Paris) presented evidence that thymus lymphocytes of newts and salamanders are of blood-borne origin. Hence, either there are fundamental differences in the pattern of lymphopoiesis among amphibia or, perhaps more likely, there is a common phylogenetic pattern involving migration of blood-borne stem cells to organs such as thymus. Volpe's results are compatible with the latter notion if stem cell migration occurs early in ontogeny and is restricted in time, as demonstrated by Le Douarin.

The source of blood-borne stem cells was also debated. It has been suggested that all haemopoietic stem cells are derived from yolk sac (Moore and Metcalfe, *Br. J. Haematol.* **18**, 279; 1970). F. Dieterlen-Lievre (Nogent-sur-Marne), however, demonstrated convincingly for the first time that definitive erythropoietic stem cells are derived from other sources. Yolk sac cells can populate the thymus, but they probably require a previous sojourn in the bone marrow (O. Stutman, New York).

The remarkable sequence of cellular and biochemical events which occurs during embryonic erythropoiesis—changing erythroid cell populations, changing haemoglobins and changing sites of erythropoiesis—was examined by several workers. J. Samarut *et al.* (Lyon) demonstrated the existence of separate erythroid stem cells specific for foetal and adult erythropoiesis respectively. Thus the situation contrasts with the synthesis of various immunoglobulin classes in B lymphocytes, which probably occurs in a single cell line (see Cooper below). J. Godet *et al.* (Lyon) identified membrane antigens specific for embryo and adult erythrocytes and A. Fantoni (Rome) showed that erythroid cells undergoing terminal differentiation in the 10–14-d mouse embryo synthesise haemoglobin by translating preformed long-lived

messengers and that ageing leads to messengers which are unsuitable for terminating translation, thus resulting in reduced haemoglobin synthesis. The role of long-lived messengers in erythropoiesis leads one to wonder whether they may not operate in lymphopoiesis—perhaps explaining how a single lymphocyte can express two immunoglobulin classes at the same time. R. A. Rifkind (New York) discussed the regulation of foetal erythropoiesis. While erythropoietin serves as a cell-specific growth regulator (? at a proerythroblast stage), the rate of terminal differentiation is controlled at a more distal step along the erythroid pathway. In addition, changes in the composition of erythroid chromatin proteins probably have an important regulatory role (V. M. Ingram, Massachusetts Institute of Technology). R. Jacquot (Reims) and O. Gallien-Lartigue (Orsay) described new observations on the effects of glucocorticoids on foetal erythropoiesis.

The ontogeny of the immune response with its important implications for the generation of diversity of responsiveness was discussed next. L. Du Pasquier (Basel) pointed out that a broad range of antibody diversity appears early in amphibian development when the animals possess a small number of lymphocytes. This interesting observation is somewhat surprising in view of the restricted responsiveness of individual lymphocytes to antigen as envisaged by clonal selection and established by experiment. M. Cooper (University of Alabama, Birmingham) and P. Kincade (New York) showed that heterogeneity of responsiveness in terms of specificity and class of antibody is generated in the avian bursa of Fabricius from day 12 of incubation. Evidence that various immunoglobulin classes are generated within a single cell line by a preprogrammed switch mechanism in the bursa is convincing. On the basis of antigen binding studies, Cooper also suggested that specific clones of antigen-binding cells develop in a fixed sequential pattern which is not influenced by exogenous antigen. He interpreted this data as favouring a model in which individual stem cells give rise to multiple clones of B lymphocytes by a predetermined pattern of sequential expression of V-region genes. P. Toivanen (Turku, Finland) also pointed to the importance of the bursa which he showed was an important source of cells able to reconstitute immunodeficient chicks.

The importance of a circulating thymic factor (TF) in T-cell differentiation was discussed by J. F. Bach (Paris). This factor has been shown to possess several biological activities including restoration of suppressor and cytotoxic T-function in thymus-

deprived mice. Its effect may be mediated by prostaglandins as well as cyclic AMP (M. A. Bach, Paris). TF seems to be one of the most functionally active factors of the various putative thymic hormones that have been described and so its further study is of considerable interest; however, the target cell for the action of these factors is as yet unclear and F. Looz (Basel) described studies with nude mice which suggested that T-cell differentiation begins at a prethymic level. Le Douarin *et al.*, however, presented evidence that stem cells which have already settled in the bursa can migrate to and differentiate in the thymus. Does this mean that stem cells which would have become B lymphocytes can instead become T cells if they are in the thymic microenvironment? Whatever the answer to these problems it is likely that various differentiation pathways are involved in the generation of T-cell subsets which were described in the thymus by M. Papiernik (Paris) and in the periphery by P. Häyry (Helsinki). Moreover, to obtain full functional maturation as described by R. Auerbach (University of Wisconsin, Madison), it seems likely that cell proliferation will be a crucial step as has been found in erythroid maturation by Rifkind.

In summary, the meeting was important in defining somewhat neglected areas for future study. In particular, most participants found the interdisciplinary approach of considerable value. □



A hundred years ago

MR. CROSS on Monday received a very numerous deputation from the British Medical Association, who laid before him their views with regard to the Vivisection Bill now before Parliament. These opinions were conveyed by Mr. Ernest Hart, Mr. John Simon, Dr Wilks, senior physician of Guy's Hospital, and Sir W. Jenner, who raised his voice against a measure which would place men of science under police supervision, and would lay a ban upon them for inflicting cruelties on the lower animals when ten thousand times greater cruelties were inflicted by those who were going to pass this Bill. Such conduct would make those who passed it objects of scorn to all the scientific men in Europe. The Home Secretary, in reply, pointed out that the Bill was framed practically in accordance with the views of the Royal Commission, and that whether the Bill passed now depended entirely upon the line of conduct pursued by the medical profession. from *Nature*, **14**, July 13, 241; 1876.

articles

Giant radio galaxy NGC315

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A giant radio source with an unusual Z-shaped structure extending over nearly one degree on the sky has been detected around the nearby elliptical galaxy NGC315. The source has structure aligned with the minor axis of the optical galaxy on linear scales ranging from a few tens of kpc to ~ 1.7 Mpc. There is also a bright compact (<48 pc) source with an opaque radio spectrum located in the galactic nucleus. The physical properties of the extended emission are compared with those of the other three well studied giant radio galaxies—DA240, 3C236 and Centaurus A. The configurations of the giant systems with respect to their parent galaxies suggest that they are not produced by a gravitational slingshot process. The unusual morphology of the source is readily explained in terms of the rotating-beam model of Henriksen and Bridle.

WE report new radio observations of the bright ($m_{pc}=12.5$ mag) elliptical galaxy NGC315 which reveal emission close to the minor axis of the galaxy over nearly 1° on the sky. This evidence suggests that it is a giant radio source some 1.7 Mpc in extent, at a distance of 100 Mpc. Only 3C236 has a linear size substantially larger than this.

Radio emission from NGC315 was first detected by Davis¹ in a 1.4-GHz survey where it was catalogued as DW0055+30. In the BDFL catalogue² of intense sources at 1.4 GHz it was included in the set of "confused fields", which have high probabilities of being multiple sources of large angular extent. Recently, Fanti *et al.*³ have presented a detailed study of the central radio source. We have mapped approximately one square degree around NGC315 at high sensitivity using the resurfaced Arecibo reflector at 2.38, 1.41 and 0.43 GHz and the Westerbork synthesis telescope at 0.61 GHz. The results confirm that NGC315 is associated with emission features at least $30'$ and probably 1° in extent. High resolution maps of the central region have also been made with the NRAO 4-element interferometer at 2.7 and 8.1 GHz.

Radio structure of the source

Figure 1 shows contours of the emission around NGC315 observed at Arecibo with the system parameters listed in Table 1. The central source coincides with the visible galaxy and is joined by a bridge of emission to the peak of an extended feature $\sim 16'$ to the north-west in position angle -48° . The central source, the bridge and this north-preceding extended feature comprise the confused region

Fig. 1 Contours of the emission around NGC315 observed at Arecibo at three frequencies. *a*, 2.38 GHz, contours at 12.5, 25, 50, 100, 200, 400 and 800 mJy per beam. *b*, 1.41 GHz, contours at 50, 100, 200, 400, 800 mJy per beam. *c*, 0.43 GHz, contours at 125, 250, 500, 1,000, 2,000 mJy per beam. The dashed lines delimit the mapped regions and the half-power beamwidths are shown as the circles in the lower right corner of each map.

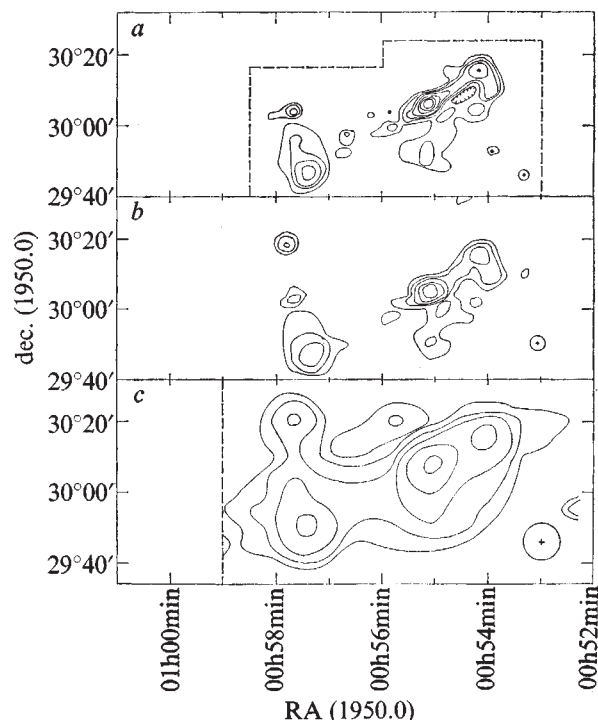


Table 1 Arecibo system parameters

Frequency (GHz)	Beamwidth (arc min)	System temperature (K)	Bandwidth (MHz)
2.38	3.0	42	20
1.41	4.0	120	10
0.43	10.0	120	2

noted by BDFL. The continuity of the radio emission from the galaxy to the extended feature leaves little doubt that all of this emission is related to NGC315. The 2.38-GHz map also clearly shows a spur of emission extending for $>15'$ SE of NGC315 towards the peak of a second emission feature located at $35'$ from the galaxy in position angle 122° . This 'spur' is also visible, though less well defined, on the 1.4-GHz map; its alignment with the south-following feature suggests that the entire emission complex is associated with NGC315.

It is unlikely that the region is confused by foreground galactic features since the galactic latitude of the source is -32° . The probability of finding two unrelated extended features of this intensity aligned with 'bridge' and 'spur' protruding in nearly opposite directions from NGC315 is also very small. Inspection of the Palomar sky survey prints of the field shows that no other galaxies of brightness comparable to NGC315 are located within the extended radio features. The 14.1-mag galaxy NGC311 at 00 h 54 min 49.6 s, $+30^\circ 00' 35''$ (L. L. Dressel and J. J. Condon, unpublished) lies near the weak emission $\sim 6'$ from NGC315 in position angle $\sim 220^\circ$, so this emission may not be related to NGC315. With this exception it is very probable that the entire extended structure shown in Fig. 1 is a single radio galaxy of exceptional angular extent.

The Westerbork telescope works at a frequency of 0.61 GHz, a synthesised beamwidth of $1' \times 2'$, a system temperature of 350 K, a bandwidth of 4 MHz, a shortest baseline of 54 m, with increments of 72 m up to 1,422 m and an average HA of cuts of -76° , -35° , -1° , $+37^\circ$ and $+77^\circ$. Observations consist of 12-min measurements made at five different hour angles. The resulting synthesised beam has radial grating responses which have been removed from the map by the usual CLEAN⁴ procedure. The map in Fig. 2 shows a strong unresolved central component with a near-in extension of $2'$ directed north-west and a longer but less intense counter component pointing south-east. Further to the south-east (near 00 h 56 min, $+29^\circ 58'$) a small feature appears which is also well aligned with the major axis of the extended central emission. A ridge in the north-preceding component running nearly transverse to the source major axis is apparent. The south-following component, though heavily resolved, becomes clearly visible when the data are convolved to a $2' \times 4'$ beam. Considering their different resolution and sensitivity, the Westerbork and Arecibo maps are in excellent agreement.

Figure 3 shows structure on a scale of $\sim 90''$ detected at

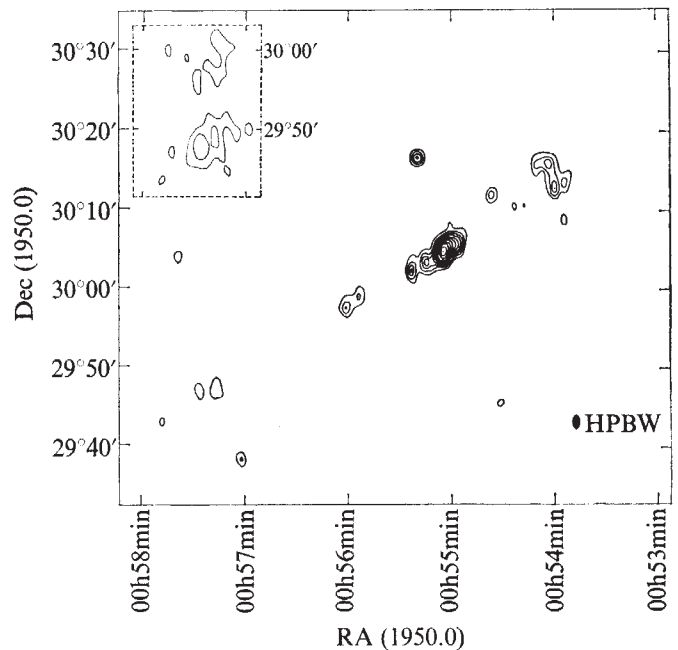


Fig. 2 Contours of the 0.61-GHz emission around NGC315 observed at Westerbork with $1' (\alpha) \times 2' (\delta)$ resolution. Contours at 25, 50, 75, 100, 150, 200, 300, 400, 500, 750 and 1,000 mJy per beam. The insert, displaced in declination only and with the same contour values, shows the south-eastern lobe when convolved to a resolution of $2' (\alpha) \times 4' (\delta)$. The maps have not been corrected for attenuation by the $1.4'$ primary beam.

2.7 GHz near the unresolved nuclear source, which has been subtracted from this map for clarity. This structure is elongated along the position angle $133 \pm 3^\circ$, and is ten times brighter north-preceding the nucleus of NGC315 than on the south-following side at this resolution. In spite of the change in frequency, the structure seems similar to that shown in the 1.4-GHz map³. The emission, whose linear scale is ~ 50 kpc, comparable with that of some ordinary radio galaxies, comprises the most intense parts of the bridge and spur detected in the measurements at Arecibo and Westerbork. It also lies within NGC315, which has a diameter of 65 kpc and whose projected minor axis of $132 \pm 4^\circ$ is close to that of the radio axis from ~ 50 kpc to 1.7 Mpc.

Comparison with other giant radio galaxies

Table 2 summarises the physical parameters of NGC315 using $z=0.0167$ (ref. 5) with the Hubble constant taken as $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The separation of the components places this radio source in the class of giant radio galaxies such as DA240, 3C236 and Centaurus A. Physical parameters* for these other sources are also given in Table 2.

As in the other giant sources, NGC315 contains a compact radio component located within the visible galaxy. An unresolved ($<0.1''$) source with an opaque spectrum

Table 2 Parameters of extended components of giant sources

	NGC315		DA240	3C236	Cen A
	North-preceding	South-following	Average	Average	Average
$\alpha(1950.0)$	00 h 54 min 11 $\pm$ 1 s	00 h 57 min 21 $\pm$ 1 s	—	—	—
$\delta(1950.0)$	$30^\circ 15' 30'' \pm 1'$	$29^\circ 46' 10'' \pm 1'$	—	—	—
Flux density (2.38 GHz)	0.55	0.45	—	—	—
Projected distance from galaxy (kpc)	450	980	600	2,700	400
Volume (kpc ³)	1.3×10^6	2.2×10^6	2.7×10^8	1.3×10^9	9.9×10^7
Spectral index α	-0.52	-0.53	-0.77	-0.60	-0.6
Luminosity (W) 10 MHz-10 GHz	6.4×10^{33}	5.2×10^{33}	1.3×10^{35}	4×10^{35}	8×10^{34}
Minimum internal energy (J)*	6.0×10^{50}	6.7×10^{50}	1.4×10^{52}	5.4×10^{52}	9×10^{51}
Equipartition magnetic field* (gauss)	1.3×10^{-6}	1.1×10^{-6}	1.5×10^{-6}	1.2×10^{-6}	1.0×10^{-6}

*Assuming equal energies in protons and electrons, and that the source volume is uniformly filled.

coincides with the nucleus of NGC315 to within the $\sim 3''$ uncertainty in the optical position (ref. 3, and L. L. Dressel and J. J. Condon, unpublished).

The physical parameters of this nuclear source are given in Table 3. We can compare the 2.7-GHz and 8.1-GHz flux densities of the nuclear source with the values tabulated in ref. 3. This shows that the spectrum flattens out between 5 GHz and 8.1 GHz, if the source has not varied.

As in the other giant radio galaxies, the outer components of NGC315 are substantially extended transverse to their axis of separation from the optical galaxy. The NRAO interferometer observations show that neither peak in the outer components contains structure $< 5''$ in extent stronger than 20 mJy at 2.7 GHz, and the Westerbork observations show there are no features $< 1'$ stronger than 60 mJy at 0.61 GHz in the south-following component. The faint extensions of the outer components show an unusual symmetry with respect to the galaxy. The more distant, south-following component subtends the same angle ($\sim 40^\circ$) at the galaxy as does the closer, north-preceding component. The outer isophotes of both components are extended almost at right angles to the line joining their peaks, but in opposite directions. As the emission bridge and spur point towards these peaks, the overall form of the structure is best described as a Z shape whose extremities are equal arcs centred on the galaxy.

The extended features of the source seem to have a spectral index $\alpha = -0.5 \pm 0.2$ with little variation across the entire source except for the nuclear component. There is some indication from the 0.43-GHz map that the northwards extension of the south-following component may have a steeper spectrum than the rest of the source, but the present data do not allow us to place firm constraints on spectral index variations with size scales $\lesssim 5'$.

Discussion

As in the giant system 3C236 (refs 6, 7 and 8) the radio emission is elongated along the projected minor axis of the optical galaxy both far from NGC315 and near its nucleus. This is at variance with the gravitational 'slingshot' model of source formation⁹ unless we make the unconventional

Table 3 NGC315—parameters of nuclear source

$\alpha(1950.0)$	00 h 55 min 05.64 $\pm$ 0.02 s
$\delta(1950.0)$	30°04' 56.8 $\pm$ 0.2''
$S_{2.7}$	0.51 $\pm$ 0.01 Jy
$S_{8.1}$	0.64 $\pm$ 0.02 Jy
Diameter	< 48 pc
Luminosity 10 MHz–10 GHz	7×10^{33} W
Minimum internal energy*	1.5×10^{46} J
Equipartition magnetic field*	$> 10^{-3}$ gauss

*With assumptions as in Table 2.

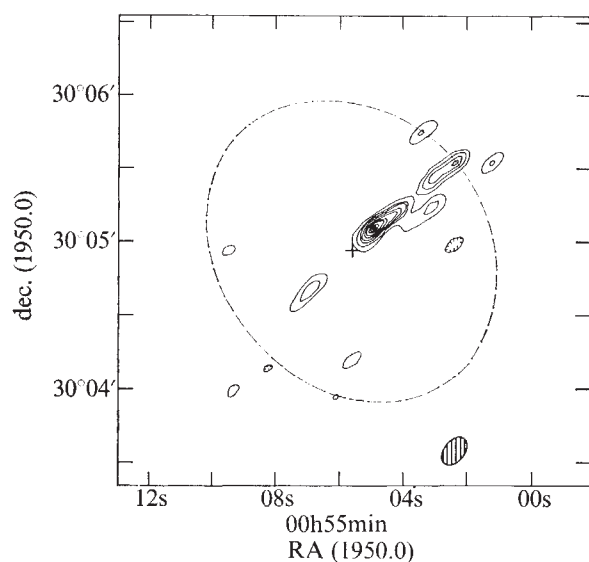
assumption that NGC315 and 3C236 are both prolate rotators, so that their minor axis lies in their rotational equators. Even then the observed alignments of their inner and outer radio structure would be statistically implausible. As the rotation axis of the giant source Cen A is known to lie near the overall elongation of both its large and small scale radio structure¹⁰, the orientations of giant radio sources relative to their galaxies seem generally to conflict with a slingshot model for their origin. This contrasts with the orientations of double radio galaxies of 'normal' dimensions, which show no strong preference for galactic minor axes^{11,12}.

The unusual Z-like configuration and rotational symmetry of the outer components with respect to NGC315 suggest that the development of a classical 'double' structure has been modified either by a mechanism acting on the components outside the galaxy or by a perturbation of the primary collimation mechanism producing the double structure. The source morphology could, for example, be produced by diffusion of particles and fields governed by asymmetries in the environments of the components such that material near the peak of the northern component can diffuse more readily towards the south, and vice versa. The fact that both components subtend the same angle at NGC315 would, however, require a specially contrived slab-like symmetry in the environment, extending over nearly 2 Mpc near the galaxy. Unless we regard the symmetrical appearance of the source as fortuitous, it is more attractive to consider an interpretation in which the primary source-collimating mechanism appears to have rotated on the sky during the lifetime of the radiating particles.

Such a rotation is implicit in the model of extended sources recently advanced by Henriksen and Bridle (to be published), in which relativistic particles are beamed from radio galaxies into double-source components along magnetic flux ropes which are rigidly coupled to the rotation of the central regions of the galaxies. In their model, the outer source components are produced where the rigidity of the rotating beams fails because their rotational velocity approaches that of light. The Z-shaped morphology of the NGC315 source can be understood on the Henriksen-Bridle model if the relativistic beams make an angle of $\sim 20^\circ$ with the rotation axis of the central region of NGC315, and the rotation rate of this region and thus of the beams is $\sim 10^{-14}$ s⁻¹. The outer extensions of the source components would then be explained as trails of particles and magnetic field left behind around the orbits of the beam ends over $\sim 10^7$ yr. As ejection of the beams near the galactic rotation axes favours the formation of giant sources, the orientational correlation observed for radio galaxies of different linear sizes is also consistent with this model. A detailed discussion of NGC315 in relation to the rotating-beam model will be given elsewhere (Henriksen and Bridle, in preparation).

It should be noted that this giant source has been recognised by the same observational procedure as were DA240 and 3C236, namely sensitive mapping of a confused field from the BDFL catalogue at resolutions of the order of a few arc min. As the BDFL confused fields are an essentially complete sample of such regions at 1.4 GHz, it is unlikely

Fig. 3 Contours of the 2.7-GHz emission near the nucleus of NGC315 observed with the NRAO interferometer, after subtraction of the bright nuclear component (see Table 3) at the position marked by the cross. The contour interval is 12 mJy/beam. The ellipse in the lower right corner indicates the half-power beamwidths, which are $11.4'' \times 4.9''$ with the major axis along position angle 138° . The dotted ellipse indicates the extent of the visible galaxy on the E print of the Palomar Sky Survey.



that radio galaxies of large angular size comprise $>5\%$ of extragalactic sources stronger than 2 Jy at 1.4 GHz, and possibly comprise substantially less than this. Although they are valuable testing grounds for theories of radio galaxies, the giant systems probably do not represent a numerically important source population that is absent from catalogues such as that of BDFL.

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Repeated sequence specific to human males

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Restriction enzyme analysis and nucleic acid hybridisation show that there is a repeated sequence in human male DNA which is not present in human female DNA. This sequence is shown to constitute about 50% of the DNA in the Y chromosome and is a simple tandemly repeated DNA.

THE contribution of DNA sequence organisation to chromosome structure and function has been the subject of much speculation¹⁻³, but there is still very little concrete evidence to relate to the theories. As a starting point, it is useful to know the location of specific sequences within the chromosomes. The technique of *in situ* or cytological hybridisation has been applied successfully to repetitive sequences such as satellite DNA, ribosomal DNA, 5S DNA and other sequences of this type⁴⁻⁶. The method is especially powerful when applied to polytene chromosomes where even single copy genes can be located. One limitation of the method is that it is designed to locate sequences which can be purified as either RNA or DNA. Different methods are needed if the aim is to analyse the composition of a particular chromosome. For this it would seem necessary to isolate the chromosome. It is difficult to purify individual chromosomes at present, but they can in many cases be separated from the rest of the complement by the formation of heterokaryons⁷. Comparative methods can then be used to analyse the sequences on the odd chromosome.

A comparable situation exists when one sex has a heterogametic sex chromosome. In principle this chromosome can be studied by comparison of male and female. The experiments reported here use this approach to study the DNA of the human Y chromosome.

Repeated sequence specific to the Y chromosome

When human DNA is digested with a number of restriction enzymes and the products analysed by electrophoresis on Agarose gels, two enzymes, *EcoRI* and *HaeIII*^{8,9}, produce prominent bands. The sizes of the *EcoRI* bands are 320 and 640 base pairs¹⁰. The band pattern with *HaeIII* is more complicated, with at least fifteen bands between 100 and 2,000 base pairs (Fig. 1). A comparison of male and female human DNAs after digestion with *HaeIII* showed that there are two bands which are found only in DNA from males. As a check DNAs from 12 individual placentae were sexed by restriction in a blind test. The correct sex was predicted

from electrophoresis of *HaeIII* digests of the DNA in all 12 cases.

The fraction of the total DNA in the male specific bands was estimated by adding known amounts of linear SV40 DNA to total human DNA which had been digested with *HaeIII* (*EcoRI* digestion of SV40 closed circular DNA was used to produce linear SV40 DNA molecules¹¹). The areas in the male specific bands and in the SV40 bands were compared by microdensitometry of photographs of ethidium bromide fluorescence¹². The peak area of the SV40 band was proportional to the amount of this DNA added to the total human DNA. By this method the larger male specific band was estimated to be $0.4 \pm 0.1\%$ (ten measurements) of the total DNA in both a placental male DNA and DNA from a male derived neuroblastoma cell line. In an identical experiment DNA from a lymphoid XYY line was found to have a large male specific band which was $0.75 \pm 0.1\%$ (five measurements) of the total DNA.

The smaller of the male specific bands is more difficult to quantify because of the higher background of other DNA sequences at this molecular weight; it constitutes about 0.2% and 0.4% of the total DNA respectively. The lymphoid XYY cell line used here has a karyotype which is near normal apart from the presence of two large chromosomes which fluoresce brightly when stained with quinacrine. Doubling the number of Y chromosomes doubles the proportion of the total DNA present in both the large and the small male specific bands, and this strongly implies that the sequence is present on the Y chromosome itself.

Differences have been found in the amount of Y chromosome fluorescence but no significant differences in the amount of the male specific bands could be detected between male DNA samples from six different placentae and four cell lines. The method is, however, not sensitive enough to detect small differences.

Fragment lengths were measured by gel electrophoresis on slab gels with marker DNAs in adjacent slots. λ CDNA restricted with *EcoRI* was used as a standard for the larger bands¹³ and *EcoRII* digested mouse satellite DNA for the smaller bands¹⁴. Band sizes are given in Fig. 1. The larger of the male specific bands is not separable from the smallest λ .*EcoRI* band when the two DNAs are analysed on the same gel slot. Its molecular weight is 2.24×10^6 , the smaller of the male specific bands has a molecular weight of 1.6×10^6 .

The human diploid nucleus contains 3.5×10^{12} daltons of DNA¹⁵. From these values, from the fraction of the DNA present in each of the two male specific bands and from

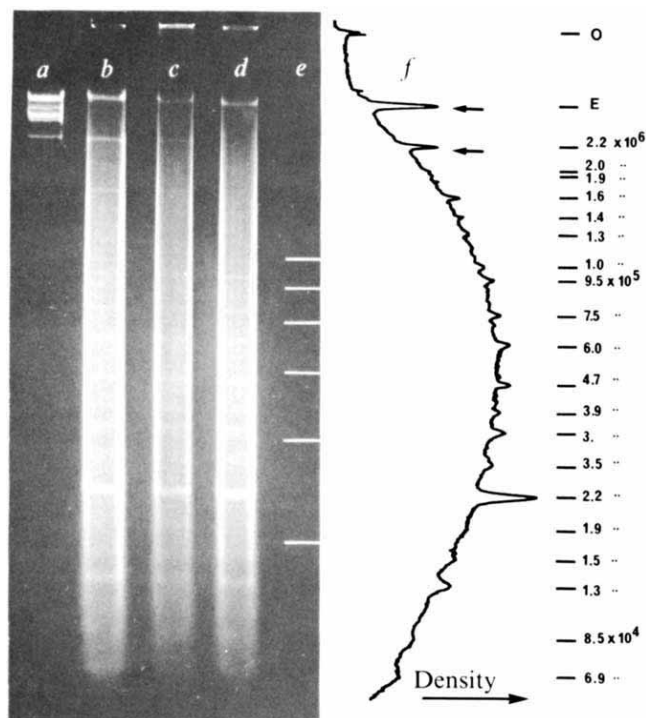


Fig. 1 DNAs restricted with different restriction enzymes after electrophoresis on a 2.5% Agarose slab gel. The gel was made up and run in 15 mM Tris, 18 mM NaH_2PO_4 , 0.5 mM EDTA (pH 7.8) with a voltage gradient of 7 V cm^{-1} for 4 h. a, 0.2 microgrammes of λ -cDNA digested to completion with *EcoRI* in 10 mM NaCl, 10 mM MgCl₂, 10 mM 2-mercaptoethanol; the DNA was heated to 70°C for 10 min after digestion to melt the sticky ends of the phage DNA. b, 10 μg of DNA from GIL cultured cells (this is a lymphoid cell line with two Y chromosomes—a gift from M. Steel). This DNA was digested to completion with *HaeIII* in the buffer as in a. c, 10 μg of DNA from a male placenta digested with *HaeIII* as in a. d, 10 μg of DNA from a female placenta digested as in b and c. e, 0.01 μg of mouse satellite DNA partially digested with *EndoR EcoRII*; the mouse satellite bands in this track were too faint to reproduce and so their positions were blocked in for this photograph. f, a trace of the pattern shown in slot b and reproduced at the same scale; the male specific bands are indicated by arrows and sizes in daltons of the principal bands given in the right hand column. O, origin of electrophoresis; E unresolved DNA.

their repeat length the number of copies of the sequences can be calculated as 6.2×10^3 for the large band, and 4.4×10^3 for the smaller band.

Restriction of fractions from a $\text{Ag-Cs}_2\text{SO}_4$ gradient followed by gel electrophoresis showed a considerable enrichment for the larger of the male specific bands on the light side of the main band (Fig. 2). The smaller of the bands was not enriched in these fractions showing that the two bands arise from different repeated sequences. This is the region of the gradients which would be expected to contain human satellites III and IV¹⁸.

Other restriction enzymes

Fractions from $\text{Ag-Cs}_2\text{SO}_4$ gradients which were enriched for the large male specific band were recycled on identical gradients to give further purification. This DNA was digested with a number of restriction endonucleases to attempt to map restriction sites within the *HaeIII* repeat length. Seven other restriction enzymes were available, *EcoRI*, *EcoRII*, *HaeI*, *HaeII*, *HindIII*, *HpaII* and *BamI*¹⁷; of these only *EcoRI* gave a distinct band. This had the same mobility as the larger *HaeIII* band, but had about one third the intensity. Double digestions with *HaeIII* and the enzymes listed above confirmed that the *HaeIII* repeat did not contain any of these restriction sites at a frequency high enough to give visible bands. The result with *EcoRI*

suggests, however, that a subset of the repeated sequence contains *EcoRI* sites at the same spacing as the *HaeIII* sites. At present it is not possible to say whether the two sites are on the same or separate molecules.

The resistance of this DNA fraction to such a wide range of restriction enzymes is to be expected since it is shown by sequence analysis described below to consist of a short repeat.

Isolation

As the 2.24×10^6 -dalton fragment is not cleaved by a large number of restriction endonucleases, restriction with these enzymes can be used to remove other sequences from the fragment partially purified by sizing. Human male DNA digested by *HaeIII* was fractionated on a preparative gel electrophoresis apparatus (E. M. Southern, unpublished) and the DNA in the size class from 2 to 2.5×10^6 daltons taken, purified by CsCl centrifugation and concentrated. This DNA was then digested with *HindIII*, *BamI* and *EcoRII* and fractionated on an Agarose slab gel. The male specific band was 70 to 80% pure as judged by microdensitometry of the gel photographs. The band was cut from the gel and the DNA recovered on to hydroxyapatite¹². Male specific DNA was isolated in this way rather than by $\text{Ag-Cs}_2\text{SO}_4$ centrifugation because larger quantities of DNA could be used; the method is also faster and less expensive than centrifugation in Cs_2SO_4 .

Homology with other sequences in male and female human DNA

It is clearly of interest to see whether the large male specific band has any sequence homology with other fractions both

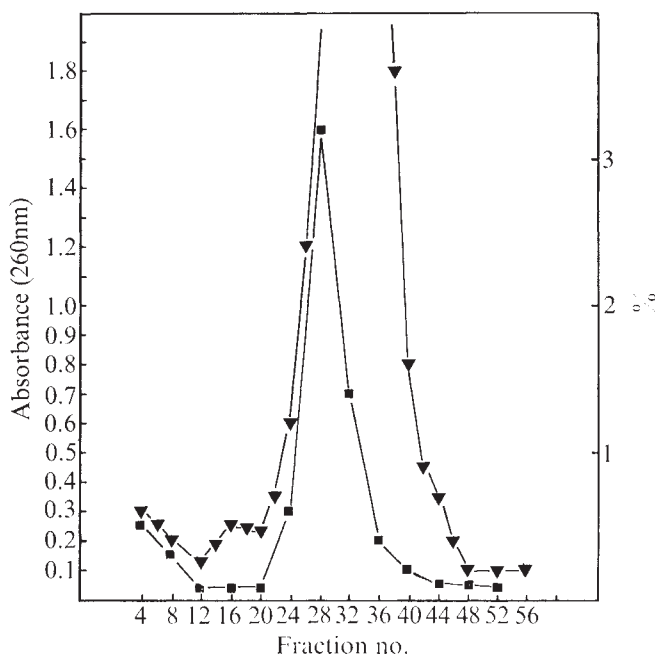


Fig. 2 Preparative $\text{Ag}^{2+}\text{-Cs}_2\text{SO}_4$ centrifugation of human male DNA from placenta was carried out essentially as in ref. 21. Human male DNA was dialysed exhaustively against 0.1 M Na_2SO_4 . Gradients were made up in 5 mM $\text{Na}_2\text{B}_4\text{O}_7$ (pH 9.3), 0.1 M Na_2SO_4 , the silver to DNA phosphate ratio was adjusted to 0.2 and the final density of the solution to 1.48 g ml^{-1} . Centrifugation was carried out in a MSE 8×40 rotor at 32,000 r.p.m. for 4 d. The gradients were fractionated with a float from the top and groups of four fractions were pooled, dialysed against 4 M NaCl and the DNA concentrated by pelleting at 30,000 r.p.m. in a MSE 10×10 rotor overnight. Each set of pooled fractions was then restricted with *HaeIII* and the products analysed by Agarose gel electrophoresis. The pattern in each slot was traced on a Joyce-Loebl recording microdensitometer and the percentage of the total area under the curve present in the male specific band calculated for each fraction: ▲, absorbance at 260 nm; ■, percentage of total DNA present in the male specific band.

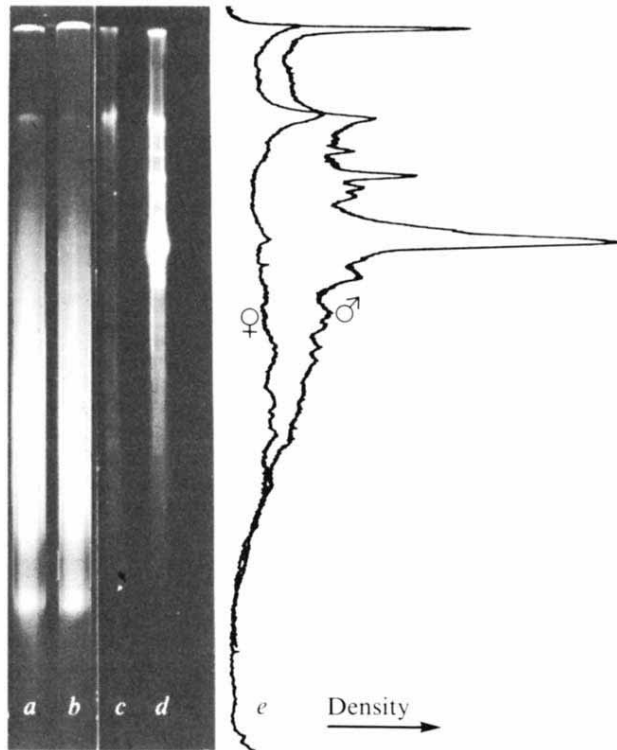


Fig. 3 Hybridisation of cRNA from the male specific band to male and female DNA. 80 μg of human male and female DNAs were digested to completion with *Hae*III then the fragments fractionated by electrophoresis on 0.8% Agarose tube gels, 1.0 cm diameter $\times$ 25 cm with a voltage gradient of 1 V cm^{-1} for 6 h. After electrophoresis the gel was photographed and the DNA fragments transferred to membrane filters as in ref. 20. Male and female DNAs were transferred simultaneously to one sheet of Millipore filter and this sheet of filter immersed for 18 h at 60°C in a small volume (about 5–10 ml) of $2\times\text{SSC}$ containing 0.01 microgrammes of ^{32}P -labelled cRNA with a specific activity of $2\times 10^8 \text{ d.p.m. } \mu\text{g}^{-1}$. After hybridisation and washing the filter was dried and autoradiographed. *a*, Gel containing female DNA before transfer to filter; *b*, gel containing male DNA before transfer to filter; *c*, negative of autoradiograph of hybridisation to female DNA; *d*, negative of autoradiograph of hybridisation to male DNA; *e*, microdensitometer trace of autoradiographs in *c* and *d*. *a*, *b*, *c*, *d*, and *e* are all reproduced on the same scale.

in male and female DNA. If, as has been suggested, tandemly-repeated sequences are involved in the meiotic pairing of chromosomes¹⁸ it would seem unlikely that sequences related to the Y specific sequence will be found elsewhere.

Absence of an homologous band in the restriction pattern is not sufficient to rule out the possibility that female DNA contains a similar sequence. First, the method would not detect less than 1,000 copies and second, bearing in mind what is known about the evolution of repeated sequences^{12,14,19}, it is possible for related sequences to give completely different restriction patterns. A more sensitive and general test for sequence homology is given by molecular hybridisation and this method is especially convincing when applied to restriction fragments separated by gel electrophoresis²⁰.

An RNA copy of the 2.2×10^6 -dalton fragment labelled with ^{32}P was synthesised using *E. coli* RNA polymerase. As judged by microdensitometry this fragment was 70% pure. *Hae*III fragments of male and female human DNA were separated on Agarose gels, denatured and transferred to strips of cellulose nitrate filter. The cRNA was then hybridised to the DNA bound to the cellulose and the strip was autoradiographed using X-ray film. The results of such an experiment are shown in Fig. 3.

As expected, male DNA gives a very intense band of hybrid at the position corresponding to 2.2×10^6 daltons, with weaker bands at positions corresponding to 4.5 and

6.7×10^6 daltons, the predicted sizes for dimer and trimer of the 2.2×10^6 dalton fragment. In addition, there are two bands between 2.2 and 4.5×10^6 daltons, and a number of indistinct bands smaller than 2.2×10^6 daltons. The male specific band which can be seen in *Hae*III restricted DNA at 1.6×10^6 daltons does not hybridise the cRNA probe used here and so is not closely related to the larger male specific sequence, in agreement with the behaviour in density gradients.

In contrast, female DNA gives a much weaker and different pattern. It shows one weak band at the position of DNA which is not cleaved by the restriction enzyme and a very faint band at the position of the larger male specific sequence. The latter is almost certainly due to sequences in the cRNA which originate from this size class of DNA and are not removed in purification of the Y specific sequence. The hybridisation to undegraded DNA occurs in both male and female and could be caused by a cross reaction to a related sequence present in both male and female DNA which does not contain the restriction site.

These results suggest that in human male DNA there is a complex family of repeated sequences related to the 2.2×10^6 -dalton DNA fragment. This complexity most probably reflects the structure of the sequences on the Y chromosome since they are not present in female DNA. The presence of dimers and trimers of the 2.2×10^6 -dalton sequence suggests that divergence is taking place in a tandemly-repeated sequence in a similar way to the *Mus musculus* and *Apodemus* satellites^{12,14}.

Fig. 4 Autoradiograph of RNase T₁ digest of ^{32}P labelled cRNA complementary to the male specific band. The cRNA was synthesised as in ref. 21 using ^{32}P -labelled GTP with a specific activity of 2 Ci mmol^{-1} . Electrophoresis and chromatography was carried out as in ref. 21, followed by chromatography on polyethylene-imine cellulose thin layers.



Fingerprint analysis

Figure 4 shows a ribonuclease T₁ fingerprint of cRNA transcribed from the large male specific band using ³²P-labelled GTP²¹. There are a number of intense spots which fall mostly in the short sequence region of the fingerprint with very small amounts of radioactive oligonucleotides present in the position of the longer sequences. The more intense spots (numbered in Fig. 4) were cut from the fingerprint and subjected to sequence analysis by pancreatic ribonuclease digestion. Table 1 gives the sequences of the spots from the ribonuclease T₁ fingerprint and the percentage of total counts in the excised spots for each sequence. Sixty-seven per cent of the total activity counted was in three sequences, AG, UG, and A₂UGG; many of the sequences present in smaller amount could be accounted for by base substitution into these sequences. Although it is not possible to derive a definitive sequence from this data it is clear that the sequence is a simple one which has diverged from a repeating oligonucleotide such as (AGUGG)_n. This is the pattern of divergence which has been found in a number of satellite DNAs^{22,23}.

Discussion

The human Y chromosome is about the same size as chromosomes 21 and 22, each of which contains about 0.82% of the absorbance of the diploid karyotype at 257 nm²⁴. On the assumption that the Y chromosome contains a similar proportion of the total DNA the two male specific bands described here would account for 70% of the DNA in the Y chromosome. The mammalian Y chromosome has been considered to be largely genetically inactive on the basis of its heterochromatic appearance²⁵. It must, however, have genetic activity since genes on the Y chromosome must switch development of the embryonic gonad towards production of a testis²⁶. In addition there seems likely to be a gene, or a function of the testis-determining gene, responsible for production of the H-Y antigen in mammals^{27,28}. These loci must either be pericentric or on the short arm of the Y chromosome in man since an isochromosome of the long arm of the Y chromosome does not cause testis formation²⁹.

At least some of the repeated sequences described above are likely to be on the long arms because of the correlation between repeated sequences and heterochromatin³⁰ or more simply because it is unlikely that the short arms could contain 70% of the Y chromosome DNA.

Four human satellite DNAs have been reported present on the Y chromosome but they are also found on a fairly large group of other chromosomes³¹. Clearly this is not the case for the sequence described here but neither the satellite DNAs nor this sequence have been sufficiently well characterised to rule out the possibility that this sequence is related to one or more of the human satellites. The lack of hybridisation to female DNA suggests that this sequence is not simply one of the satellite DNAs but the behaviour in Ag-Cs₂SO₄ density gradients suggests that it is possible that satellite I, III and IV could be contaminated with this sequence. This might explain some of the *in situ* hybridisation of these satellites to the Y chromosome.

The conclusion which is inescapable from the *in situ* hybridisation and the experiments reported here is that the human Y chromosome must contain very little apart from simple sequence DNA.

The functions of repeated and satellite DNAs remain unknown in spite of much work on the structure and organisation of this class of DNA sequences. There are two questions raised by the data reported here which have obvious significance in terms of repeated sequence function; why is there so much repeated sequence DNA on the Y chromosome and why is the sequence described here found only on the Y chromosome? One answer to the first question could

Table 1 Sequences of spots from ribonuclease T₁ fingerprints

Spot no.	% Counts	Composition	Sequence
1	5.5	G	G
2	33.6	AG	AG
3	2.3	CG	CG
4	5.8	A ₂ G	AAG
5	0.58	ACG	ACG
6	1.32	ACG	CAG
7	0.25	C ₂ G	CCG
8	16.9	UG	UG
9	5.5	AUG	AUG
10	3.0	CUG	CUG
11	16.9	A ₂ UG	A ₂ UG
12	2.66	C ₂ UG or A ₃ G	A ₃ G
13	1.9	U ₂ G	U ₂ G
14	2.5	U ₂ AG	U ₂ AG
15	0.87	—	—

Spots on the fingerprint in Fig. 4 were located by autoradiography, cut from the thin layer and counted in toluene base scintillator. After counting, the oligonucleotides were eluted, digested with ribonuclease A and analysed by electrophoresis as in ref. 21.

be that since the male-determining genes must segregate during spermatogenesis they must be present on a chromosome. If these genes are coded by small amounts of DNA then perhaps it is necessary to add other DNA to produce a chromosome. The repetitive sequences may be such genetically neutral DNA.

A further consequence of the need to ensure that genes on the Y chromosome segregate is that no crossing-over with other chromosomes can be tolerated³². This requirement could provide the answer to the second question above if the presence of a set of sequences unique to the Y chromosome prevented chiasmata formation with other chromosomes. The X and Y chromosomes in man have a terminal association during diakinesis and first meiotic metaphase³³ which involves the short arms of both chromosomes^{34,35} but which does not have the normal form of synaptenemal complex and at meiosis they form a special structure as in most mammals³⁶. The Y specific sequences could either be involved in this behaviour or could be a result of it.

The genetic isolation of the Y chromosome might allow a unique set of repeated sequences to be produced either by saltation²² or by sister chromatid exchanges³⁷. The formation must have been fairly recent since DNA from male chimpanzees shows no homologous band in the restriction pattern with *Hae*III (K. W. Jones, personal communication). It is of course possible that related sequences without the restriction site are present.

The availability of RNA complementary to a human Y specific sequence should allow further study of the evolution and distribution of these sequences. Clearly this type of study can be extended to repeated sequences of other chromosomes by the use of DNA from mouse/human somatic cell hybrids which contain only a single human chromosome.

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Note added in proof: A human Y specific sequence has been purified by extensive hybridisation of male and female DNA (ref. 38). The authors found that there is a repeated sequence DNA which constitutes between 2 and 11% of the human Y chromosome. This sequence is most likely to be one of the sequences described here. The major difference between the results reported in this paper and in ref. 38 is in the amount of the Y specific sequence. Calculations of the amount of sequences by Kunkel *et al.*, are rather indirect and assume that all Y specific repeated sequences are recovered at each step of purification. Cumulative losses in the purification procedure could thus account for

an underestimate of the quality of Y specific repeated sequences present in the human genome.

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Initiation sites of Rous sarcoma virus RNA-directed DNA synthesis *in vitro*

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Rous sarcoma virus DNA synthesis in disrupted virions is initiated mainly at a site about 200 nucleotides or less from the 5' terminus, but other initiation sites throughout the RNA seem to be used as well. No AUG triplet occurs within a 5' terminal segment of about 25 nucleotides.

THE RNA genome of avian oncornaviruses consists of two¹ identical RNA strands, each with a molecular weight of about 3×10^6 (about 10,000 nucleotides)^{2–4}. During the early stages of the infective cycle the viral genome is transcribed into a complete double-stranded DNA copy of molecular weight 6×10^6 (ref. 5). When DNA is synthesised *in vitro*, however, in partially disrupted virus particles, the product is short, averaging only a few hundred nucleotides (see review by Green and Gerard⁶). Nonetheless, a small percentage of the DNA strands can reach a length of 1,500 to 4,500 nucleotides⁷ or even the length of the viral RNA⁸. The short size of the bulk of the product is believed not to be due to nucleolytic scission of the DNA, since short DNA was also synthesised in reconstituted systems devoid of detectable nuclease activity^{7,9}. A substantial fraction of the *in vitro* DNA product is complementary to a small region of the viral RNA^{10,11}, although sequences complementary to virtually all the viral RNA are present^{10,12}.

Several groups have reported that most or all DNA generated *in vitro* is attached to the 3' end of a unique species of RNA primer^{13,14} (tRNA^{Trp} in the case of Rous sarcoma virus RNA (ref. 15)) and that only one molecule of this primer is associated with each viral RNA subunit¹⁶. It therefore seemed reasonable to assume that DNA synthesis started close to the 3' end of the RNA template and that premature chain termination gave rise to incomplete DNA strands with the properties described above. Recently, however, Taylor and Illmensee¹⁶ reported that DNA transcription begins very close to the 5' terminus of the RNA. This conclusion raised the question as to how sequences complementary to almost all the RNA are synthesised and how long DNA strands are generated.

We have examined the origin and the complexity of the DNA synthesised by disrupted Rous sarcoma virus, Prague B strain (Pr-B RSV), in the following way. The DNA was annealed with Pr-B RSV ³²P-RNA, and the hybridised RNA was characterised by digestion with RNase T₁ and identification of the resulting

large oligonucleotides. The location on the viral RNA of the regions complementary to the cDNA could be determined from the physical map of large T₁ oligonucleotides of Pr-B RSV RNA established in our laboratory^{17,18}. It was found that the most frequent DNA species corresponds to a region which is not more than 200 nucleotides in length and includes the 5' terminus of the RNA. Several other non-contiguous regions of the viral RNA, notably one located in the middle of the molecule, were also represented in varying amounts. These findings suggest that the major initiation site is located near the 5' terminus but that DNA synthesis may also originate at various other positions throughout the genome.

RSV ³²P-RNA complementary to RSV cDNA

DNA synthesised by partially disrupted Pr-B RSV particles in the presence of actinomycin D was purified and hybridised to Pr-B RSV ³²P-RNA. Hybridisations were carried out at two concentrations of DNA, the lower one to hybridise preferentially the more prevalent DNA species and the higher one to ascertain the overall complexity of the DNA. The preparations were treated with RNase T₁ and the RNase-resistant fractions were isolated by chromatography on Sephadex G-100. At the lower DNA concentration 4.7% of the RNA was hybridised and at the higher concentration 13.2%. A control which had been annealed to globin cDNA yielded 2.0% of the input radioactivity (mainly due to poly(A), see below) in the hybrid fraction. The same quantity of ³H-labelled 70S Pr-B RSV RNA was then added to each sample as an internal standard to monitor the recovery of radioactivity throughout the subsequent analysis. The mixture was denatured and digested to completion with RNase T₁, and the products were separated by two-dimensional polyacrylamide gel electrophoresis.

A standard fingerprint pattern of 70S Pr-B RSV RNA is shown in Fig. 1a; the characteristic, large T₁ oligonucleotides which are numbered in the tracing (Fig. 1a') have been characterised, and their relative order on the genome has been determined^{17,18}. Figure 1b shows the oligonucleotides recovered after hybridisation at the lower DNA concentration: only two of the characteristic large T₁ oligonucleotides appeared as intense spots, namely T₁₃ and to a lesser extent T₁. The identity of these oligonucleotides was ascertained from their pancreatic RNase digestion products (data not shown). Three further spots, T₅₁, T₅₂ and T₅₃, were also prominent. These fragments

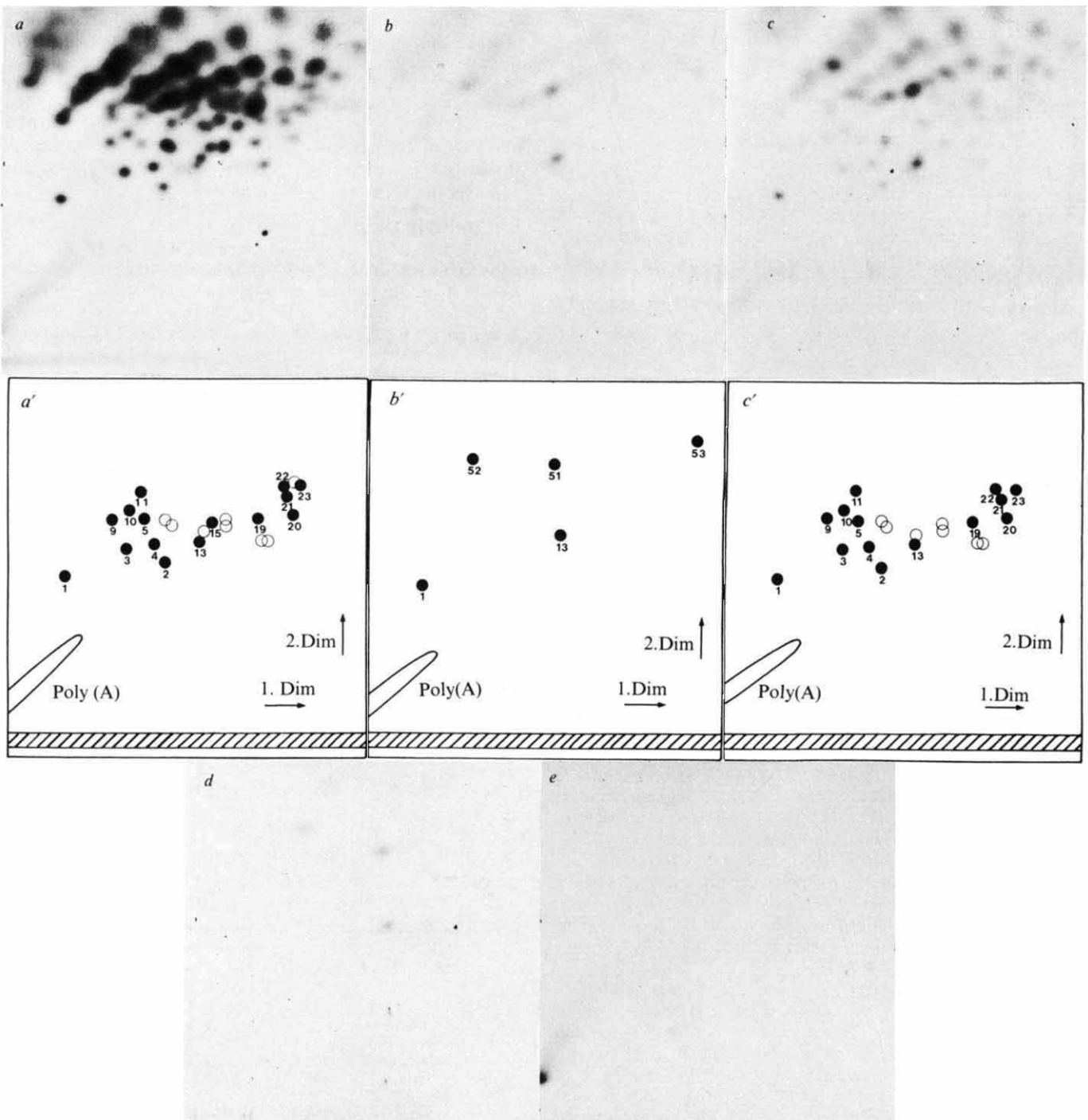


Fig. 1 Two-dimensional polyacrylamide gel electrophoresis of T_1 digests of Pr-B RSV ^{32}P -RNA recovered after hybridisation to different cDNA preparations. Rous sarcoma virus, Prague B strain (Pr-B RSV), was grown on chicken fibroblasts (Spafas, C/O, chf⁻, obtained from R. Friis) as described¹⁷, with the following modifications. Infected cells were passaged four or five times in Petri dishes and then seeded into Corning tissue culture roller flasks (490 cm², No. 25130) at a density of 3×10^7 cells per flask in 50–100 ml of Dulbecco's modified Eagle medium–10% Tryptone phosphate broth–5% calf serum. The virus was collected every 24 h and stored at -70°C . Pr-B ^{32}P -RSV was prepared in roller bottles essentially as described¹⁷. Synthesis of Pr-B RSV DNA: Virus from 1 l of collected medium was concentrated by centrifugation for 60 min at 4°C and 28,000 r.p.m. in the S30 Spinco rotor and suspended in 1 ml of 100 mM NaCl–50 mM Tris-HCl (pH 7.5)–1 mM EDTA. The concentrated virus suspension was incubated with 1 μl of β -mercaptoethanol and 0.3 ml of 1% NP 40 (w/v) for 5 min at 37°C . The mixture was adjusted to 38 mM Tris-HCl (pH 8.0), 28 mM NaCl, 4.8 mM MgCl₂, 0.09 mM each dATP, dTTP, dCTP, and ^{32}P -dGTP (specific activity 3×10^3 c.p.m. nmol⁻¹), 24 μg ml⁻¹ of actinomycin D (final volume, 2.1 ml) and incubated for 60 min at 37°C . The preparation was then digested with 0.25 mg ml⁻¹ of Pronase in the presence of 0.5% SDS for 15 min at 37°C . After phenol extraction the combined aqueous phases were adjusted to 1% sodium acetate (pH 5) and precipitated with 2.5 vol of ethanol. After 2 h or longer at -20°C , the precipitate was collected by centrifugation, redissolved in 250 μl of 50 mM NaCl–20 mM Tris-HCl (pH 8)–5 mM EDTA–0.1% sodium dodecylsulphate and chromatographed through a 0.6×8 cm column of Sephadex G-100 equilibrated with the same buffer. The excluded material was precipitated with ethanol, the precipitate dissolved in 100 μl of 0.2 M KOH, and digested for 16 h at 37°C . The digest was neutralised and chromatographed through a 0.5×8 cm column of Sephadex G-100 in 20 mM Tris-HCl (pH 8)–0.1 M NaCl. The excluded material was precipitated with ethanol as above and dissolved in 20 mM Tris-HCl (pH 7.5)–5 mM EDTA. The yield in different preparations ranged from 0.3 to 1.5 nmol of incorporated dGMP. Analytical or preparative zonal centrifugation of the viral DNA was through a 4-ml 5–23% sucrose gradient in 50 mM Tris-HCl (pH 7.5)–0.2% SDS for 120 min at 55,000 r.p.m. and 15°C in the SW60 Spinco rotor. A parallel tube was run with ^{125}I -labelled globin mRNA, Friend cell ribosomal ^3H -RNA and *E. coli* tRNA. Annealing reactions: 70S Pr-B RSV ^{32}P -RNA (specific activity $> 10^6$ c.p.m. μg^{-1}) was denatured in 20 mM Tris-HCl (pH 7.5)–5 mM EDTA for 1 min in a boiling water bath. Each annealing reaction mixture contained 2×10^6 c.p.m. of the denatured Pr-B RSV ^{32}P -RNA, DNA as indicated below, 0.5 M NaCl, 0.01 M Tris-HCl (pH 7.5) in 100 μl and was incubated for 4 h at 66°C . The solution was diluted to 0.25 M NaCl (200 μl) and digestion carried out with 4 U of RNase T₁ (Calbiochem) for 30 min at 37°C in the presence of 25 μg of yeast RNA (purified by phenol extraction). After phenol extraction the aqueous phase was chromatographed on a 1×16 cm column of Sephadex G-100 in 0.5 M NaCl–0.01 M Tris-HCl (pH 7.5). To the pooled excluded fractions were added QB RNA (100 μg) and 70S Pr-B RSV ^3H -RNA (2.5×10^5 c.p.m., specific activity $> 10^6$ c.p.m. μg^{-1}) as carrier and internal standard, respectively. The nucleic acids were precipitated twice with ethanol, taken up in 6 μl of 20 mM Tris-HCl (pH 7.5)–2 mM EDTA, sealed in a capillary, heated for 1 min in a boiling water bath and chilled. The RNA was digested with 16 U of RNase T₁ in 10 μl of the same Tris-EDTA buffer for 30 min at 39°C . The products were subjected to two-dimensional polyacrylamide gel electrophoresis as described¹⁷. The autoradiograms are of the following samples: *a*, Total T₁ digest of untreated Pr-B RSV ^{32}P -RNA, directly subjected to two-dimensional polyacrylamide gel electrophoresis; *b*–*e*, were processed as described above; the DNA in the annealing mixtures was as follows: *b*, Pr-B RSV DNA, equivalent to 120 pmol of dGMP; recovery of ^{32}P radioactivity in hybrid, 4.2%; *c*, Pr-B RSV DNA equivalent to 500 pmol of dGMP; recovery in hybrid, 13.2%; *d*, Pr-B RSV DNA, equivalent to 80 pmol of dGMP; *e*, rabbit globin cDNA, $s_{20,w}$ about 9S (obtained from Dr Peter Curtis), 0.2 μg . *a*–*c*' are tracings of *a*–*c*; *b*' also identifies the spots in *d*. The hatched bar in the tracing indicates the position of the gel strip from the first dimension.

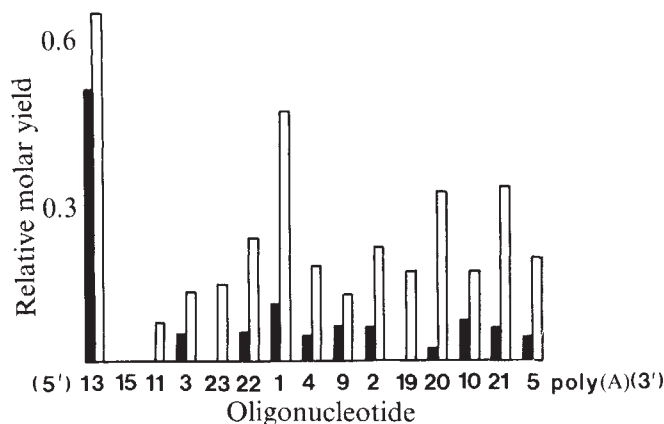


Fig. 2 Relative yields of some large T_1 oligonucleotides from Pr-B RSV ^{32}P -RNA recovered after hybridisation to Pr-B RSV RNA. The gel regions corresponding to the large T_1 oligonucleotides shown in the autoradiographs of Fig. 1b and c were excised, and the ^{32}P radioactivity (due to the hybridised RNA) and the ^3H radioactivity (due to the total Pr-B RSV RNA added as internal standard) were determined as described¹⁷. The relative recoveries were calculated by multiplying the $^{32}\text{P}/^3\text{H}$ ratio of each sample by a factor which takes into account the nucleotide composition of the particular oligonucleotide and the distribution of ^3H label between C and U, as described in the legend to Table 2 of ref. 17. The values (in arbitrary units; chosen to give approximately the estimated absolute yields) are plotted in the order of the oligonucleotide map as determined by Joho and Stoll (unpublished revision of the earlier map of Coffin and Billette¹⁷). Solid bars represent the yields obtained after annealing at low, and open bars at high, DNA concentration.

had not been analysed or mapped previously since they are found in a gel region densely populated with smaller oligonucleotides. Figure 1c shows the fingerprint of T_1 oligonucleotides recovered after hybridisation at the higher DNA concentration. Again, T_{13} and T_1 were the strongest of the large and T_{51} , T_{52} and T_{53} of the smaller T_1 oligonucleotides; in addition almost all characteristic T_1 oligonucleotides were present, albeit with varying degrees of intensity. When globin-specific cDNA was substituted for RSV DNA the autoradiogram revealed the presence of poly(A) and some faint spots corresponding to smaller T_1 fragments but no large oligonucleotides (Fig. 1e). Thus, even the longest oligonucleotides generated by T_1 digestion of non-hybridised RSV RNA are not excluded by Sephadex G-100.

To measure the recovery of the individual oligonucleotides, the appropriate gel disks were excised, and the ^{32}P radioactivity as well as the ^3H radioactivity (from the internal standard) were determined. The relative molar yield of each ^{32}P -oligonucleotide was calculated by multiplying the ratio of its $^{32}\text{P}/^3\text{H}$ radioactivity by a factor which takes into account the base composition of the oligonucleotide and the distribution of ^3H label between UMP and CMP (see legend to Fig. 2 of ref. 17). The relative molar yields of selected large T_1 oligonucleotides which were well resolved on the autoradiogram and had been mapped earlier^{17,18} (E. Stoll and R.H.J., unpublished results) are given in Fig. 2. Oligonucleotide T_{13} , which is recovered in highest molar yield (about 65% of the theoretical amount), had been mapped as the oligonucleotide closest to the 5' end and, as shown below, in fact constitutes the 5' terminus. Oligonucleotide T_1 , which occurs in the next highest yield, is located in the middle of the map. After hybridisation at the lower DNA concentration the molar yield relative to T_{13} was 20% for oligonucleotide T_1 and 10% or less for all other nucleotides. At the higher DNA concentration the recovery of oligonucleotide T_1 was 70% compared with T_{13} while most other large oligonucleotides were present at 20 to 50%. Interestingly T_{15} and T_{11} , the two oligonucleotides closest to T_{13} , were barely detectable.

A similar protection experiment was performed with Pr-B RSV DNA fractionated on a neutral sucrose density gradient.

Two fractions, one corresponding to a sedimentation coefficient of 0.4S and the other of 4.14S, were used for hybridisation. In both cases T_{13} was by far the most prominent of the large oligonucleotides, followed by T_1 ; T_{51} , T_{52} and T_{53} were again the most intense of the smaller oligonucleotides (data not shown). Figure 1d shows the result of an experiment in which the product recovered after hybridisation to cDNA was of strikingly low complexity; no spots besides T_{13} , T_{51} , T_{52} and T_{53} were present at a significant intensity.

Oligonucleotide T_{13}

Oligonucleotide T_{13} was digested enzymatically and the products were analysed. As shown in Table 1, pancreatic RNase digestion yielded, among other nucleotides, a product Xp (previously thought to be AAAC from its electrophoretic mobility^{17,18}) which was not susceptible to further degradation by the combined action of RNases A, T_1 and T_2 . This mixture yields mononucleotides from normal oligonucleotides. This resistance to digestion was reminiscent of the behaviour of the so-called capping group $m^7\text{G}^{5'}\text{ppp}^{5'}\text{G}^{\text{m}}\text{pCp}$ found at the 5' terminus of RSV RNA by Keith and Fraenkel-Conrat¹⁹ and Furuichi *et al.*²⁰. To compare Xp with the authentic capping group, 30–40S Pr-B RSV ^{32}P -RNA was digested exhaustively with pancreatic RNase A, RNase T_1 and RNase T_2 , and the digestion products were separated by two-dimensional electro-

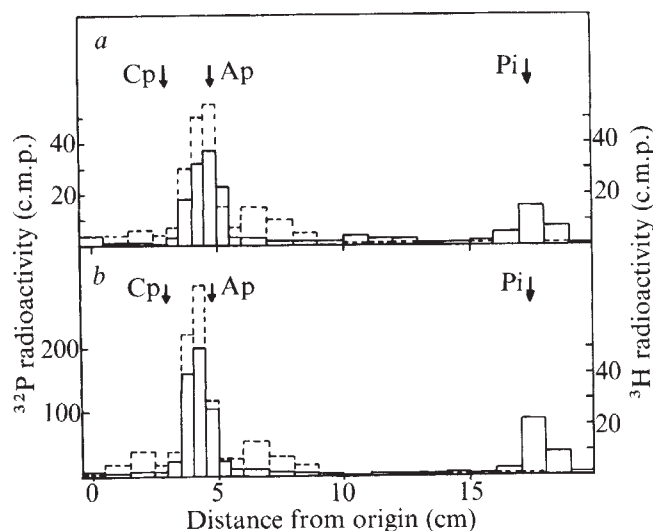


Fig. 3 Coelectrophoresis of ^3H -methyl- $m^7\text{G}^{5'}\text{ppp}^{5'}\text{G}^{\text{m}}\text{pCp}_{\text{OH}}$ and the dephosphorylated ^{32}P -labelled putative capping group derived from oligonucleotide T_{13} and from 30–40S Pr-B RSV RNA. ^{32}P -labelled oligonucleotide T_{13} (7,000 c.p.m.) (a) and Pr-B RSV ^{32}P -RNA (3.4×10^6 c.p.m.) (b), prepared as described in the legend to Table 1 were digested in 10 μl of 7.5 mM sodium acetate (pH 4.5)–0.5 mM EDTA with RNase A (0.8 mg ml^{-1}) and a Takadiastase extract (containing RNase T_2 and T_1 , about 80 U ml^{-1} , see ref. 32) for 2 h at 38 °C in the presence of 40–50 μg of yeast carrier RNA. The products were separated by two-dimensional electrophoresis as described by Adams and Cory²¹. In the case of (a) as well as of (b) a product (Xp and X'p respectively) with a mobility relative to Gp of 1.2 in the first, and 0.12 in the second, dimension was identified, in addition to the four standard nucleotides. X'p (990 c.p.m.) and Xp (225 c.p.m.) were eluted, mixed with 10 μg of yeast RNA and digested with 0.018 U of alkaline phosphatase from *E. coli* (Worthington) in 10 μl of 50 mM Tris-HCl (pH 8.8)–10 mM MgCl_2 for 60 min at 38 °C. The digests were mixed with about 1 A_{260} unit each of (3')AMP and (3')CMP, 0.1 μmol of unlabelled inorganic phosphate and ^3H -methyl- $m^7\text{G}^{5'}\text{ppp}^{5'}\text{G}^{\text{m}}\text{pCp}_{\text{OH}}$ (about 2,000 c.p.m.; the preparation was a gift of Drs Furuichi and Shatkin) and subjected to electrophoresis on Whatman 3MM paper at pH 3.4 ^{32}P -phosphate was run in a parallel lane and located by autoradiography. The standard nucleotides were located in the ultraviolet light. Each lane, (a) and (b), was cut into strips which were placed in scintillation vials with 10 ml of toluene-based scintillator solution. ^3H radioactivity (— — —) and ^{32}P radioactivity (———) were determined by scintillation counting. Background radioactivities (5 c.p.m. for ^3H , 9 c.p.m. for ^{32}P) were subtracted from each value.

Table 1 Digestion products of oligonucleotide T₁₃

RNases T ₂ +T ₁ +A	Pancreatic RNase A	RNase U ₂
0.82 Xp	0.8 Xp	0.8 X-C-Ap
5.4 Ap	3.0 A-Up	1.0 U-U-U-U-Ap
1.0 Gp	2.7 A-Cp	0.82 U-(C,U)-Ap
6.8 Cp	5.1 Cp	0.83 U-U-Gp
7.3 Up	5.1 Up	1.6 C-C-Ap
	1.0 Gp	1.0 C-Ap

Pr-B RSV ³²P-RNA (20×10⁶ c.p.m.) was digested with RNase T₁, the products were separated by two-dimensional polyacrylamide gel electrophoresis and oligonucleotide 13 was extracted as described in ref. 17. Aliquots (1,000–3,000 c.p.m.) were digested with RNase U₂, pancreatic RNase A or with a mixture of RNases T₂, T₁ and A (for base analysis) as described²¹. The digestion products were separated at pH 3.5 on Whatman 3MM paper for base analysis and on DEAE paper in the other cases. The products of U₂ digestion were characterised by their mobilities and/or further digestion as for base analysis. The yields of all products were measured by scintillation counting. Molar compositions are relative to the underlined nucleotides; Xp, identified as the capping group (see text) contains five phosphate residues.

phoresis as described by Adams and Cory²¹. Oligonucleotide T₁₃ was subjected to the same treatment in parallel. Pr-B RSV RNA yielded almost exclusively the four standard mononucleotides and about 0.042% of a product X'p with electrophoretic properties similar to those described for the capping group²¹. The mobility relative to Gp in the first dimension was 1.2, in the second 0.12; the corresponding values of Adams and Cory²¹ were 1.1 and 0.092. Oligonucleotide T₁₃ gave the four standard mononucleotides and 22.5% of a product Xp which had the same relative mobility as X'p. X'p and Xp were treated with phosphatase and subjected to one-dimensional electrophoresis. In both cases 22% of the radioactivity were released as inorganic phosphate (1.1 mol), and the residual oligonucleotides X'OH and XOH had the same electrophoretic mobility as authentic ³H-CH₃-labelled.

m⁷G⁵'ppp⁵G^mpC_{OH} was obtained from Dr A. Shatkin (Fig. 3). Digestion of XOH and X'OH with snake venom phosphodiesterase yielded inorganic phosphate and products with mobilities characteristic of 7mpG, pG and pC (data not shown). These experiments prove that oligonucleotide T₁₃ contains the capping group and thus constitutes the 5' terminus of Pr-B RSV RNA. From the data of Table 1 the partial sequence m⁷G⁵'ppp⁵G^m-C-C-A-(U₄A;U(C,U)A;2C₂A;CA)-U-U-Gp was deduced. Evidently the initiation codon A-U-G is not present within the first 25 nucleotides of Pr-B RSV RNA. It is of interest to note that in RAV-6 RNA the same capping group is associated with a large T₁ oligonucleotide of a somewhat different mobility which, with one exception, yields the same products as T₁₃ on digestion with pancreatic and U₂ RNase and thus probably has a similar overall structure.

Initiation sites

We have demonstrated, by a new experimental approach, that a large proportion of the DNA synthesised *in vitro* by disrupted oncornavirus particles is complementary to a short segment of the viral RNA which contains the 5' terminus, and a smaller proportion is complementary to almost all of the RNA sequences^{10–12}. Our results confirm and extend the findings of Taylor and Illmensee¹⁶. These authors reported that the newly synthesised DNA, when isolated as a template-primer-product complex, is associated only with close to full-length, poly (A)-linked RNA and concluded that initiation occurs close to the 5' end of the RNA. We found that a major fraction of *in vitro* cDNA with a sedimentation coefficient of 4S and less, that is, with chain lengths of certainly less than 200 nucleotides, hybridises to the 5' terminal oligonucleotide (T₁₃) of Pr-B RSV RNA. The fact that the complexity of the RNA protected by the DNA is quite low, as judged by the appearance of only one large T₁ oligonucleotide in high yield and the virtual absence of the two

T₁ oligonucleotides closest to the 5' terminal fragment (T₁₁ and T₁₅), argues against the possibility that the short size of our product was due to endonucleolytic cleavage (see also refs 7 and 9). Thus, the major initiation site must lie within 200 nucleotides from the 5' terminus of the RNA, and the short size of the DNA could be explained if the 5' end of the RNA constituted a termination point for a large part of the product transcribed *in vitro*.

DNA complementary to an RNA segment located in the middle of the genome was present at a level about 20% of the major DNA species, as judged by the relative amounts of oligonucleotides T₁ and T₁₃ recovered after hybridisation at low DNA concentration. DNA sequences corresponding to other regions of the RNA, in particular close to the 3' end, occurred each at less than 10% of the major species since in the hybridisation at high DNA concentration the characteristic large oligonucleotides were recovered with a yield half or less that of T₁. The occurrence of short cDNA species, corresponding to RNA segments located throughout the genome and present in significantly different amounts, suggests that DNA synthesis is initiated at not only one but more likely several additional sites throughout the RNA. Multiple initiation sites for cDNA synthesis have also been proposed by Rothenberg and Baltimore²² for the murine leukaemia virus system. This proposal runs counter to the previous findings that all DNA synthesised *in vitro* initiates with the same nucleotide sequence at the 3' end of a unique tRNA^{13,14,15} associated with the 5' end of 36S viral RNA¹⁶. The results were obtained, however, in the presence of only three deoxyribonucleoside triphosphates, that is, under conditions of limited synthesis, which might favour initiation at the main initiation site. Furthermore, in our experiments viral cDNA was synthesised in the presence of actinomycin D to suppress synthesis of double-stranded DNA^{23,24}. cDNA synthesised in the presence of this drug (or of Mn²⁺) has a higher complexity than that produced in its absence¹²; perhaps actinomycin favours the utilisation of minor initiation sites.

If DNA synthesis initiates at the 5' terminal region by the addition of deoxynucleotides to tRNA^{Trp}, how does DNA synthesis originate at the other minor initiation sites? It has been assumed that the position defined by the 3' terminus of the primer tRNA hydrogen-bonded^{25–27} to viral RNA constitutes the attachment site for the RNA-dependent DNA polymerase. Panet *et al.*²⁸, however, have shown that the enzyme has a strong affinity for free tRNA^{Trp}; it is thus conceivable that a preformed polymerase-tRNA complex may initiate at many different sites, albeit with low efficiency. Alternatively (or in addition) a low level of initiation could occur at sites defined by the many other tRNAs^{13,28,29} associated with 70S RNA, or possibly at the 3' termini of accidental nicks in the 70S RNA, which could serve as primer template.

We do not know to what extent the *in vitro* findings with disrupted virus particles reflect the events which lead to the formation of a complete viral DNA transcript *in vivo*. If we assume, however, that the physiological initiation site is in fact located close to the 5' terminus then one must postulate a transcription mechanism that allows polymerase molecules to continue elongation at the 3' terminus, either of the same or a second molecule of the viral RNA. A model in which this feature is postulated was proposed by Cooper and Wyke³⁰. Recently, it has been reported^{7,8,22} that in appropriate reaction conditions the length of a small fraction of the cDNA synthesised by disrupted viral particles approaches that of the viral RNA. It will be of interest to ascertain whether DNA initiated at a site closer to the 3' terminus is elongated extensively or whether DNA beginning close to the 5' terminus is efficiently elongated across the (possibly ligated) gap between a 5' and a 3' terminus.

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Proviral sequences of baboon endogenous type C RNA virus in DNA of human leukaemic tissues

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Hybridisation of RNA from a baboon endogenous type C RNA virus to DNA from tissues of leukaemic patients indicates that a virus of this type is horizontally transmitted among humans. DNA from several patients with leukaemia hybridised 70% of the hybridisable RNA from baboon endogenous type C RNA virus (BaEV) and yielded hybrids of high t_m , whereas DNA from normal human tissues hybridised only 23% of the BaEV RNA, and the t_m of these hybrids was lower.

SEVERAL studies link type C RNA viruses to human leukaemia. These include the detection of subviral components within blood leukocytes taken from leukaemic patients^{1–11}, the transient release of virus-like particles from these cells in short term culture^{12,13} and the rare isolation of infectious virus from cultured human cells^{14–19}. Nevertheless, evidence for the viral aetiology of human leukaemia or, in fact, for the presence of oncornaviruses in humans has been limited by the inability to detect in fresh human tissues one component expected of virus-infected cells, namely, a DNA provirus related to a major portion of the genome of a type C RNA virus²⁰.

The detection of a DNA provirus requires the availability of an appropriate viral nucleic acid for use as a detection probe. Early studies suggested that Rauscher mouse leukaemia virus (MuLV_R) might yield a useful probe^{8–11}, but subsequent work indicated that the MuLV_R-related RNA sequences detected in leukaemic blood leukocytes might also exist in the blood cells of normal people⁴ and that these sequences might be more closely related to that of the primate RNA tumour virus, simian (woolly monkey) sarcoma-leukaemia virus (SiSV)^{4,6,7}. So far, neither RNA probes from SiSV nor from MuLV_R have been used successfully to detect DNA proviruses in human tumour cells⁴.

The isolation of infectious virus from cultured human cells has provided a new impetus in the search for provirus in human cells. In one instance^{14,15} the viruses obtained from cultured human leukaemic cells consisted of a mixture of two type C viruses; one closely related to SiSV and one closely related to

viruses obtained from apparently normal baboons, the baboon endogenous virus, BaEV^{21–24}. This result prompted us to examine various tissues from leukaemic patients for BaEV-related provirus. Evidence for BaEV-related provirus has very recently been reported in DNA from the spleen of the one particular patient, patient HL23, with acute myelogenous leukaemia (AML)²⁵ whose cells in culture had released virus, the HL23 virus (HL23V)¹⁴. We present here detailed hybridisation results that demonstrate the presence of BaEV-related provirus in seven of the leukaemic tissues studied and its absence from normal tissues. Specifically, these leukaemic tissues were chosen because of earlier evidence for the presence of other viral components in them. We also show that DNA sequences distantly related to BaEV were found in both normal and leukaemic humans.

BaEV sequences in human DNA

Figure 1 shows that more of the sequences in RNA from BaEV hybridised to DNA from spleens or peripheral blood leukocytes of some patients with myelogenous leukaemia than to DNA of tissues obtained from normal humans. DNA from some leukaemic patients (HL23, HL49) hybridised faster to BaEV RNA than did DNA from other patients but by a C_{ot} of 2×10^4 (200 h) DNA from all of the leukaemic patients presented in Fig. 1 hybridised significantly more BaEV RNA than did DNA from normal humans. It was not possible to extend the hybridisations longer than 200 h because of RNA degradation (dashed line, Fig. 1). DNA from human NC37 cells infected by BaEV should contain one provirus per haploid genome^{23,24}, and in this case BaEV sequences were readily detected (Fig. 1). Since the hybridisation conditions used detected DNA sequences present in one or more copies per haploid genome, the low hybridisation obtained with DNA from normal humans, relative to leukaemics, suggests that the sequences in the leukaemic DNA do not result from amplification of normal human chromosomal genes. In fact, the BaEV sequences in the DNA of some leukaemic patients (HL7, HL11) were probably present less than once per cell, as judged by the rate of hybridisation with BaEV RNA ($C_{ot}^{1/2} \sim 10^4$) (Fig. 1).

The experiments shown in Fig. 1 used a DNA : ^{125}I -RNA ratio of about $2 \times 10^7 : 1$. To determine whether this ratio represented an excess of BaEV-related DNA sequences, hybridisations were carried out with varied DNA : RNA ratios ($C_0t = 2 \times 10^4$) (Fig. 2). The percentage ^{125}I -RNA hybridised to DNA against the DNA : RNA ratio is shown in Fig. 2a. The results indicate that even at the highest DNA : RNA ratio examined, the percentage of the RNA hybridised was still increasing. The data of Fig. 2a were therefore subjected to a double reciprocal analysis and the resulting curves were extrapolated to the Y axis (where RNA : DNA approaches 0, DNA : RNA approaches ∞) (Fig. 2b), to estimate the fraction of BaEV RNA that may hybridise at infinite DNA excess. The results (maximum percentage of the BaEV RNA hybridised at infinite DNA) shown in Table 1 demonstrate that DNA from some leukaemics hybridised 70% of the hybridisable BaEV RNA whereas DNA from normal human tissues hybridised only 23% of the RNA at DNA excess. Most extrapolated values were close to actual values obtained with large (1 mg for example) DNA inputs; where this was not the case, the actual values obtained were used (values in parentheses). BaEV sequences in the DNA of one patient (HL54) were detectable by DNA titration analyses and especially by the double reciprocal representation (Fig. 2b), although they were not detected in kinetics experiments. The analysis of DNA from four tissues of normal persons (Table 1) establishes the lack of BaEV sequences in the normal human genome. The possible existence of some normal tissues with BaEV sequences or many normal tissues with very rare BaEV sequences (less than one copy per cell) is not evaluated here and would indicate infection of normal individuals.

Lack of detectable SiSV proviral sequences

As we have noted, some biochemical studies have indicated that leukaemic cells may sometimes contain molecules related to components of woolly monkey (simian) sarcoma-leukaemia virus, SiSV (refs 1-7, 13-19, 25, 28-30) and four laboratories have reported the release of infectious virus related or identical to SiSV from cultured human cells¹⁴⁻¹⁹. The case of patient HL23 (refs. 14-16, 25) involved release of an SiSV-related virus in addition to a virus closely related to BaEV.

In earlier work^{1,5}, however, it was not possible to detect proviral DNA related to the genome of SiSV in leukaemic patients. Since the DNA titration experiments detected BaEV sequences in DNA from leukaemic patients, this assay was applied to RNA from SiSV. Figure 2c nevertheless shows that no proviral sequences related to SiSV could be detected in tissues of patient HL23. DNA from normal humans hybridised very little of the SiSV RNA at finite DNA inputs, relative to

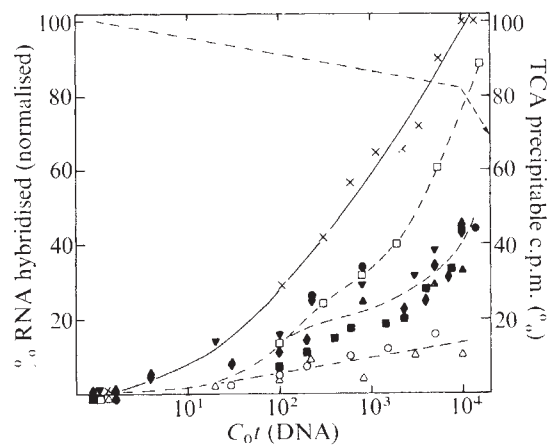


Fig. 1 Kinetics of hybridisation of BaEV RNA to cell DNA. Hybrids were formed between ^{125}I -RNA from BaEV (strain BAB8-K(FCf2TH)⁹ (500-1,000 c.p.m.; 10^8 c.p.m. μg^{-1}) and a 1×10^7 - or 2×10^7 -fold weight excess of cell DNA at 60°C in 0.4 M phosphate buffer and monitored by resistance to RNase as detailed elsewhere²⁰. Fifty-five percent of the RNA hybridised to DNA from normal baboons and all values have been normalised to this result. $\times$, Yellow baboon spleen (*Papio cynocephalus*); $\circ$, NC37 human lymphoid cultured cells derived from a normal donor; $\square$, NC37 cells infected by BaEV (NC37(BaEV)); $\bullet$, blood leukocytes of patient HL49, a 32-yr-old male Caucasian with CML (acute phase), a white blood cell (WBC) count of $1.4 \times 10^9 \mu\text{l}^{-1}$ —treatment records were not available; $\blacktriangledown$, blood leukocytes of patient HL50, a 62-yr-old male Caucasian with AML, a WBC count of $2.5 \times 10^9 \mu\text{l}^{-1}$ —untreated; $\blacktriangle$, spleen taken at autopsy of patient HL23, a 51-yr-old female Caucasian with AML and under treatment with cytosine arabinonucleoside, vincristine, and prednisone; $\blacklozenge$, blood leukocytes from patient HL7, a 26-yr-old male Negro with CML (acute phase), WBC count of $6 \times 10^9 \mu\text{l}^{-1}$, and treated with prednisone and vincristine; $\blacksquare$, spleen taken at autopsy from patient HL11, a 59-yr-old male Caucasian with an unusual aggressive form of CMML and treated with hydroxyurea; $\triangle$, liver taken from a normal human; — — —, acid-precipitability of RNA. HL, human leukaemic; AML, acute myelogenous leukaemia; CML, chronic myelogenous leukaemia; CMML, chronic monomyelocytic leukaemic.

the amount of BaEV RNA that was hybridised (Fig. 2c). Although sequences very distantly related to SiSV RNA may exist, inordinate amounts of DNA would be required for the formation of stable hybrid structures. It seems, therefore, that if the human leukaemic cells are infected by SiSV, the infection does not involve the classical mechanism of inserting one or more proviral DNA sequence per transformed cell. It remains possible, however, that provirus related to SiSV will be found in other patients.

Table 1 Maximum hybrid yield and t_m with BaEV RNA

Source of DNA	Disease	Maximum % RNA hybridised	$t_m(^{\circ}\text{C})$	$\Delta t_m(^{\circ}\text{C})$
HL23 spleen	AML	70	76	-1
HL50 leukocytes	AML	70	77	0
HL49 leukocytes	CML-A	70	78	+1
HL7 leukocytes	CML-A	(70)	79	+2
HL11 spleen	CMML	45	80	+3
HL54 leukocytes	AML	(40)	NT	—
HL51 spleen	AML	(30)	NT	—
HL55 leukocytes	ALL	(23)	NT	—
Normal human spleen	—	23	NT	—
Normal human liver	—	23	69	-8
Normal human blood leukocytes	—	23	NT	—
NC37 normal human lymphoid cell line	—	23	69	-8
NC37 (BaEV)	—	100	77	0
Normal baboon spleen	—	100	75-78*	—
Human leukocyte ($\times$ rRNA) (control)	—	—	82	—

The maximum percentage of the BaEV RNA that hybridised to DNA was estimated from Fig. 2b. t_m Values were measured as described in the text. Leukocytes, peripheral white blood cells; AML, acute myelogenous leukaemia; CML-A, acute phase of chronic myelogenous leukaemia; CMML, a case of aggressive chronic monomyelocytic leukaemia. The HL numbers correspond to those in earlier publications (refs 1-7, 14-16, 25; see also legends to Figs 1 and 2); NC37(BaEV), NC37 infected by BaEV. NT, not tested.

*Value varied with different species of baboon.

Thermal stability of hybrids formed with BaEV RNA and DNA from leukaemic patients

The thermal stability of hybrids formed at 60 °C was measured by exposure to a given temperature for 5 min in 0.15 M NaCl and then for 2 h at 37 °C in 0.45 M NaCl with RNase. Representative results are shown in Fig. 3 and are presented in entirety in Table 1. The thermal transitions of all BaEV RNA hybrids examined were broad. The t_m values of hybrids formed with DNA from positive leukaemic tissues were higher than that of hybrids formed with DNA from normal human tissues. They approached t_m values observed with hybrids formed between rRNA and cell DNA and, in fact, were higher in some instances than the t_m of hybrids formed with RNA of BaEV and DNA from normal baboons. Thus, whereas the BaEV-related sequences in DNA of normal baboons and human leukaemic tissues seem to be heterogeneous, they are equally closely related to RNA of BaEV. In contrast, the sequences in the DNA of normal human liver or NC37 cells differ more in nucleotide sequence from BaEV RNA and are therefore distinct from the more closely BaEV-related sequences in the spleens of leukaemic patients HL11 and HL23 and in the blood leukocytes of patients HL7, HL49, and HL50 (Table 1).

Phylogeny of BaEV RNA

Acceptance of the conclusion that the human leukaemic tissues contain BaEV DNA sequences is obviously contingent on the

source of the ^{125}I -RNA and the DNA used. The genetic origin of the BaEV RNA could be tested by hybridising the RNA to DNA of selected primates. These experiments were not complicated by contamination with cell RNA since the BaEV used was obtained from canine thymus cells and therefore any contaminated cell RNA would be non-primate. On the basis of the data of Benveniste and Todaro^{23,24} BaEV RNA would be expected to hybridise best to animals most phylogenetically related to baboons, and we found it to be so (Fig. 4). The data are presented as a double reciprocal analysis of DNA titration experiments using DNA from selected primates. The BaEV RNA was most closely related to DNA from baboons, of 18 Old World monkeys tested to date (Fig. 4 and L. Donehower, R. C. G., F. W.-S., and D. G., in preparation). The phylogenetic data and the hybridisation to proviral DNA in NC37 (BaEV) cells (Fig. 1) confirm that the ^{125}I -RNA was the labelled genome of BaEV.

Typing of DNA

This laboratory uses DNA from many non-human primates. Although obviously a very remote possibility, it was conceivable that the results described here were due to the selective, accidental contamination of the leukaemic tissues with primate tissues, with baboon tissue for example. An assay was devised therefore to ensure that the putative DNA preparations from human leukaemics were, in fact, human DNA. A specific ^{125}I -DNA probe was prepared from the repeated sequence populations of baboon and human DNA using the rationale of Rice²⁶ (Table 2). The baboon DNA probe had two useful properties: (1) in reconstruction experiments it detected very low amounts of baboon DNA (1%) in solutions of human DNA; and (2) it contained a very specific sequence component present in some members of the subfamily *Cercopithecinae* (baboons, macaques, and mangabeys) that was completely absent from other members of the same subfamily (guenons) as well as from other primates (other Old World monkeys, apes, prosimians, New World primates) (Table 3 and our unpublished data). Hybridisation experiments using the specific sequences from the DNA of baboons and humans and the unlabelled DNA preparations examined here showed that the human leukaemic DNA samples lacked baboon-specific sequences (Table 2), establishing that the hybridisation results obtained with BaEV RNA could not be explained by contamination of the human leukaemic DNA preparations with baboon DNA.

Interpretation of molecular hybridisation results

We conclude that five of the eight human leukaemic cases presented here had DNA sequences related to a major portion of the BaEV genome and that these sequences were absent from the normal human genome. In three of these cases, HL7, HL49, and HL50, DNA was prepared from fresh, unfrozen cells in 1974 and 1975. Aliquots of cells from these patients were stored frozen and DNA was again prepared from them in 1976. They were again positive and showed the same hybridisation kinetics as with DNA from the unfrozen cells. Therefore, the detection of BaEV proviral DNA in the positive patients was reproducible. Two other cases, patients HL51 and HL54, may also be positive (Table 1), but there was not enough DNA available for t_m analyses. The most plausible explanation of the molecular hybridisation findings is that the BaEV sequences were introduced from without by a viral agent and thus they merit the designation 'proviral' DNA sequences.

In summary, the supporting evidence is as follows. Kinetic, stoichiometric (DNA titration), and thermal stability measurements all indicated the presence of BaEV sequences in DNA from some human leukaemic tissues. Kinetic analyses showed that most of the BaEV proviral sequences in leukaemic DNA were infrequent ($C_0t_{1/2} > 10^4$), probably present at 0.1–1 copy per haploid genome. Stoichiometric studies showed that up to 70% of the hybridisable sequences in BaEV RNA were hybridised by DNA from spleens or blood leukocytes of leukaemic

Table 2 Hybridisation of type-specific ^{125}I -DNA to cell DNA

Source of unlabelled cell DNA	Source of ^{125}I type-specific DNA Baboon (<i>P. cynocephalus</i>) % Hybridisation of subfamily-specific ^{125}I -DNA (± 10)	Human
Human leukaemic tissues		
HL23 spleen	0	118
HL50 leukocytes	0	110
HL7 leukocytes	3	95
HL54 leukocytes	8	96
HL11 spleen	5	106
HL49 leukocytes	0	118
Human normal leukocytes	0	100
Normal primate tissues		
Yellow baboon spleen (<i>P. cynocephalus</i>)	100	0
Mandrill baboon brain (<i>P. sphinx</i>)	93	4
Macaque muscle (<i>Macaca mulatta</i>)	91	—
Sooty mangabey spleen (<i>Cercocebus atys</i>)	95	7
De Brazza guenon spleen (<i>Cercopithecus neglectus</i>)	0	—
Colobus (<i>Colobus abasinicus</i>)	0	0

DNA purified from tissues of a baboon (*P. cynocephalus*) and fragmented to a chain length of about 500 nucleotides was labelled with ^{125}I (3×10^7 c.p.m. μg^{-1}) by W. Prenskey. It was self-annealed in 0.14 M PO_4 to a C_0t of 5 at 70 °C. Reannealed DNA was bound to hydroxyapatite (HAP) at 80 °C, unannealed DNA was washed from the HAP at 80 °C, and reannealed DNA was eluted at 100 °C. The ^{125}I -DNA eluting between 80 and 100 °C was mixed with a 10^5 -fold weight excess of unlabelled genomic DNA from Old World primates, incubated at 100 °C to denature any double-stranded DNA, annealed at 80 °C for 15 min, and hybridised ^{125}I -DNA was purified on HAP as described above. ^{125}I eluting between 80 and 100 °C was scored as hybrid. Of the hybridisable ^{125}I -baboon DNA, two-thirds was specific for baboons, mangabeys, and macaques and one-third was less specific sequences which were conserved among all Old World primates tested, excluding prosimians. The more specific sequences were used for constructing Table 2. The units presented are percentages of "type-specific" hybridised relative to that hybridised to *P. cynocephalus* DNA and were reliable within ± 10 units ($\pm 10\%$ of the maximal value). The rationale for the experiment was that repeated sequences originating after man and baboon diverged from a common ancestor would be present in one of the animals and would be totally lacking in the repeated sequence fraction of the other. It has developed that these sequences are present in the fraction of genomic sequences that reanneal by a C_0t of 1.5 (repeated some 1,000 times) and that have a high thermal stability.

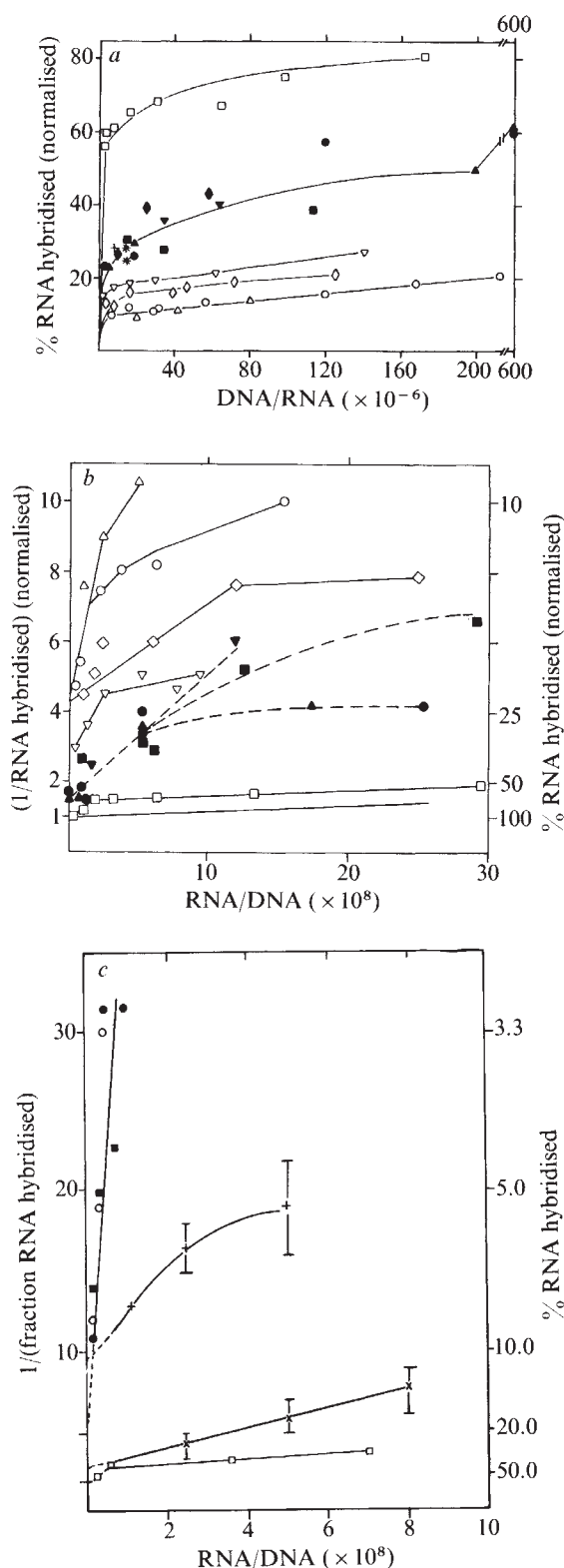


Fig. 2 Stoichiometry of hybridisation of BaEV RNA and SiSV RNA to cell DNA (DNA titration). Hybrids were formed between ^{125}I -RNA (10^8 c.p.m. μg^{-1}) and cell DNA at 60°C in 0.4 M phosphate buffer and a C_0t of 2×10^4 . Hybrid formation was monitored by resistance to RNase. *a* and *b*, Hybridisations with BaEV RNA. $\circ$, NC37 human lymphoid cultured cell line derived from a normal donor; $\square$, NC37 cells infected by BaEV; $\bullet$, blood leukocytes from patient HL49; $\blacktriangledown$, blood leukocytes from patient HL50; $\blacklozenge$, spleen from patient HL23; $\blacktriangledown$, blood leukocytes from patient HL7; $\blacksquare$, spleen from patient HL11; $\triangle$, liver from a normal human; $*$, liver from patient HL51, a female Caucasian with AML and a white blood cell count of $5 \times 10^9 \mu\text{l}^{-1}$, other data not available; $+$, spleen from patient HL51; $\diamond$, leukocytes from patient HL55, a 12-yr-old female Caucasian with ALL, other data not available; ∇ , blood leukocytes from patient HL54, a 30-yr-old male Caucasian with AML.

patients, compared with 23% hybridised by DNA from tissues of different normal humans, including liver, spleen, fresh blood leukocytes, and a cultured lymphoid cell line (NC37). The absence of the extra BaEV-related sequences in any one such tissue demonstrates that they are not Mendelian genes in normal humans and therefore do not arise in leukaemic DNA by amplification of normal human genes. Thermal stability measurements showed that among the BaEV-related sequences in the leukaemic tissues were some more closely related to BaEV RNA than any detected in normal human DNA. More specific conclusions, that is, the percentage mismatching, are premature because the DNA sequences involved seem to be heterogeneous, including the multiple BaEV sequences in normal baboons, and the t_m values obtained are an average of the stabilities of all of the RNA-DNA hybrids formed. Of the three measurements used, the DNA titration assay was the most sensitive for detecting BaEV proviral sequences.

Possible horizontal transmission of BaEV among humans

Though the sequences in human leukaemic DNA clearly qualify as BaEV sequences in the three molecular hybridisation assays described above, it was nonetheless initially surprising to find that a baboon type C virus, particularly a virus closely related to the one obtained from *Papio cynocephalus* (yellow baboon) had entered humans. In retrospect the concept is consistent with the following facts. (1) A BaEV-related P30 protein has been reported in human cells, including human leukaemic cells²⁸⁻³⁰. Although this was interpreted as expression of a human endogenous virus, which in theory may be related to BaEV, infection by exogenous BaEV itself is also consistent with the observed results. (2) Cross-species transmission of type C RNA viruses is now a well documented event^{23,24,27}; in fact, one of the cases involved transmission of an ancestral BaEV to cats, probably in the Mediterranean basin^{23,24}. (3) Although probably all Old World monkeys carry endogenous type C RNA viruses, only from baboons has the virus been isolated^{21,22}. Thus, baboons seem to release BaEV with relative ease from many tissues. The virus is not completely controlled by its host and so is a candidate for infection between species. (4) BaEV grows readily in human cells. (5) *Papio cynocephalus* and its closely related kin, *Papio anubis* (dogface baboon), are found in Africa and could have had contact with primitive man. Once BaEV entered man it could then be transmitted with or without an intermediate vector among the human population (a phenomenon which we propose is occurring today). It seems that the transfer to man occurred recently since cases where cross-species transmission of type C RNA viruses apparently occurred more than 5 Myr ago resulted in more genetic change of the viruses isolated from the new host^{23,24}. This is in contrast to the minimal differences between the BaEV RNA genome and the BaEV sequences in DNA of human leukaemics, or the sequences in the RNA of the BaEV component of HL23V.

The virus we call HL23V is similar and possibly identical in one of its two major components to BaEV^{14,15}. It was repeatedly isolated from cultures of marrow and blood cells from patient HL23, with acute myelogenous leukaemia¹⁶.

—, *P. cynocephalus* spleen. ALL, acute lymphocytic leukaemia. Results are normalised to the maximum hybridisation value obtained with DNA from *Papio cynocephalus* (55% of the RNA). *c*, Hybridisations with SiSV RNA. Hybrids were formed as described for *a* and *b*, but using ^{125}I -RNA from SiSV produced by NC37 cells. $\blacksquare$, NC37 normal human lymphoid cultured cell; $\square$, NC37 cells infected by SiSV (NC37(SiSV)); $\circ$, kidney from patient HL23; $\bullet$, spleen from patient HL23. For direct comparison, the results of *b* with BaEV RNA hybridised to normal (+) or leukaemic ($\times$) human DNA are superimposed. Only the leukaemics described by solid symbols in *b* were used. The bars represent the high and low values obtained at each RNA:DNA ratio in *b*. The results of *c* are not normalised. Note the differences in scales from *b*.

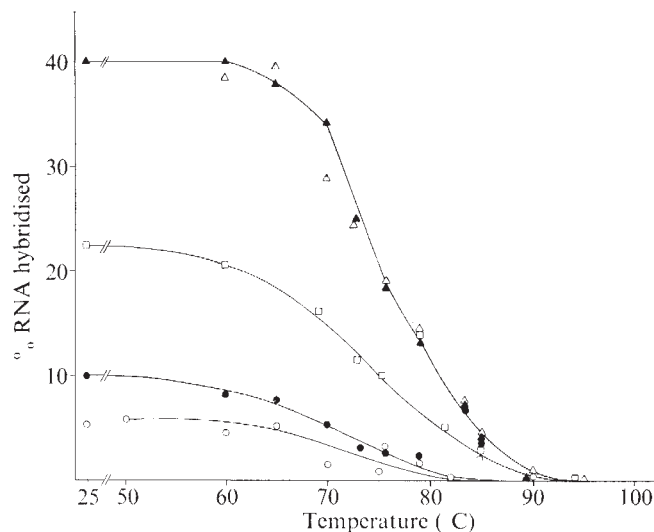
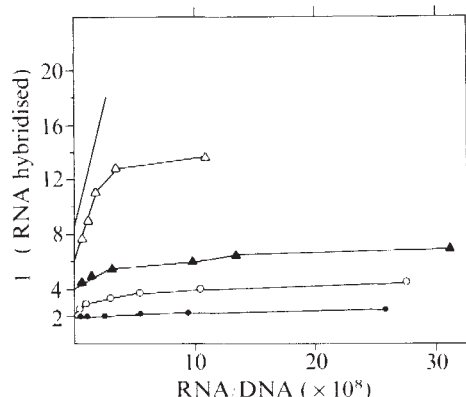


Fig. 3 Thermal stability of BaEV RNA-cell DNA hybrids. Hybrids were formed between ^{125}I -BaEV RNA and a 5×10^6 -fold weight excess of cell DNA at 60°C in 0.4 M phosphate buffer and a C_0t of 2×10^4 . The melting of the hybrid was accomplished as outlined in the text and in ref. 25. The t_m values for Table 2 reflect melting of half of the hybrids resistant to incubation at 60°C . ●, NC37 normal human lymphoid culture cell line; ○, liver from a normal human; □, spleen of patient HL23; ▲, spleen of *P. cynocephalus*; △, spleen of *P. amabis*.

The DNA purified from the spleen of this patient contains BaEV sequences (ref. 25 and this article) and the cytoplasm of the patient's fresh, uncultured, blood leukocytes contained virus-like particles with BaEV-related nucleic acids²⁵. It will be of considerable interest now to see whether the RNA of this BaEV-related virus will detect more proviral sequences in the DNA of many human leukaemic tissues than did the RNA of the BaEV isolated from baboons.

It is also of interest that another patient with BaEV provirus (HL51) was a close contact of patient HL23, a woman who presented with AML slightly before HL23 and who ultimately died of the disease. That both leukaemic contacts had BaEV provirus could be significant. Though all the tissues examined in this study were from patients who were found earlier to possess other types of viral components^{1-7,15,25}, and most of them were positive for BaEV sequences, a more random survey of leukaemic patients is in progress and results obtained to date suggests that only about 20% of patients with myelogenous and

Fig. 4 Stoichiometry of hybridisation of BaEV RNA to DNA from primates. See legend to Fig. 2 for details. ●, *P. cynocephalus* spleen; ○, sooty mangabey thymus; ▲, De Brazza guenon spleen; △, woolly monkey spleen; —, liver from normal human. Values are not normalised.



lymphocytic leukaemia contain BaEV provirus in their blood leukocytes (N. Miller *et al.*, in preparation). We feel that BaEV may not be the only type C virus infecting the human population. Our results should be interpreted in the context of the detection of: (1) proviral sequences distantly related to Rauscher mouse leukaemia virus in DNA of some patients with leukaemia or lymphomas (G. Aulakh, D. G., and R. C. G., unpublished); (2) 'extra sequences', some related to RNA of Rauscher mouse leukaemia virus, in DNA of blood leukocytes of several patients with leukaemia but missing in DNA of blood leukocytes from normal persons^{10,11,31}; (3) RNA related to Rauscher mouse leukaemia virus^{8,9}, Moloney mouse leukaemia virus³², and simian sarcoma-leukaemia virus in blood leukocytes of some leukaemic patients (A. Tavittan, personal communication); (4) antibodies in humans directed against viruses of the HL23V-SiSV-gibbon ape leukaemia virus and BaEV families^{33,34} and, less frequently, different antibodies directed against feline and mouse leukaemia viruses³⁴; and (5) antigens in tissues of some humans related to those of baboon endogenous virus²⁸⁻³⁰, simian sarcoma-leukaemia virus^{29,30}, and Friend mouse leukaemia virus³⁵. Apart from such unlikely explanations as the discarding of particular DNA sequences during normal differentiation, and their selective retention in some leukaemic people, information storage in RNA of normal cells, or unstable episomal DNA sequences³⁶, the more likely possibility is that many RNA tumour viruses originating in animals are being horizontally transmitted among humans. Whether they are pathogenic in humans remains to be established, but the majority of reports suggesting the absence of type C RNA virus components in tissues of normal humans (but see ref. 4) suggests that their potential pathogenicity in humans should be considered. In any case, we feel the present results establish the presence of type C RNA virus information in humans.

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letters to nature

Identification of Abell Cluster 754 with the X-ray source 3U0901-09 by Ariel V

THE faint, high galactic latitude X-ray source 3U0901-09 (ref. 1) has been previously associated², on positional grounds, with the rich cluster of galaxies, Abell 754 (distance class 3, richness class 2)³. Since the Uhuru 90%-confidence positional error box for 3U0901-09 is relatively large, having an area of 2.6 degree², it is worthwhile, in view of current theoretical interest in X-ray emission from clusters of galaxies (see, for example, ref. 8), to reduce the positional uncertainty and thus confirm or reject the proposed identification with A754.

The high energy (2-18 keV) detector system of the Ariel V X-ray sky survey instrument (SSI) has observed 3U0901-09 on a number of separate occasions over a period of a year (February 1975-February 1976) and the results are reported here. The SSI and data analysis are described elsewhere⁴⁻⁶.

The SSI measured a statistically significant signal ($>3\sigma$) from the vicinity of 3U0901-09 for nine sets of observations. The individual error boxes ('lines of position') from each set of SSI observations are combined to produce the Ariel V Sky survey 90%-confidence probability contour

for the source location, shown in Fig. 1. This reduces, by over an order of magnitude, the uncertainty in the location of the X-ray source and supports the previous identification² of 3U0901-09 with A754. Our 90%-confidence error box has centroid (in celestial coordinates (degrees, 1950.0)) $\alpha = 136.55$, $\delta = -9.56$, and area 0.11 degree². We have paid particular attention to the possibility that A761 might also be a source of significant X-ray emission; careful examination of the individual observations, however, allows us to reject this possibility.

Each observation consisted of a summation of data over a period of 1-3 d. There is no significant intensity variation between the individual measurements (maximum deviation from the mean is $\sim 1\sigma$), and the mean intensity level is 1.9 ± 0.2 Ariel V counts s⁻¹ (2-18 keV), corresponding to $\sim (9.5 \pm 0.9) \times 10^{-11}$ erg cm² s⁻¹ (2-10 keV) or 5.5 ± 0.5 Uhuru counts s⁻¹ (2-6 keV), assuming a spectrum like that of the Coma cluster. The uncertainties quoted are from Poisson errors on the count rates only. This is in close agreement with the Uhuru measurement^{1,2} of 4.4 ± 0.8 Uhuru counts s⁻¹. A redshift $z = 0.0537$ for A754 (ref. 7) and a Hubble constant of 55 km s⁻¹ Mpc⁻¹ give a distance of 293 Mpc, implying, from the Ariel V intensity, an X-ray luminosity of $(9.8 \pm 0.9) \times 10^{44}$ erg s⁻¹ (2-10 keV).

It has been suggested that cluster X-ray luminosity can be positively correlated with: (1) cluster richness^{9,10}; (2) cluster radio luminosity⁹; (3) the presence of one or more dominant central galaxies (usually optical type cD (ref. 11))^{9,10}; possible explanations have been discussed in the literature^{9,10,12}. Confirmation of A754 as an X-ray source strengthens these apparent correlations, A754 being both a cD type cluster¹¹ and a radio source⁹. Several theoretical studies of possible X-ray emission mechanisms in clusters of galaxies (see those reviewed in ref. 8), have suggested (differing) correlations between cluster X-ray luminosity and velocity dispersion Δv . It is therefore important to measure Δv for A754.

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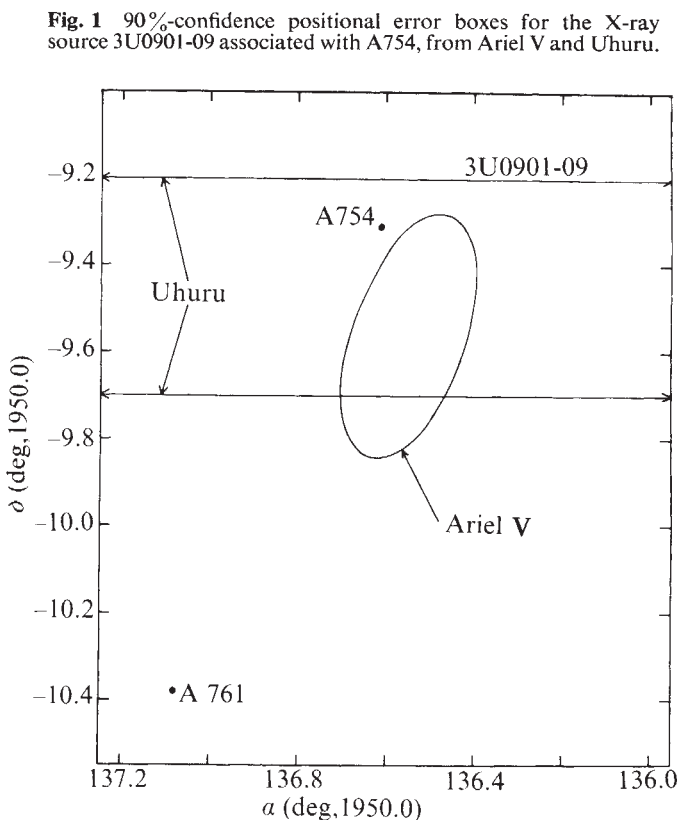


Fig. 1 90%-confidence positional error boxes for the X-ray source 3U0901-09 associated with A754, from Ariel V and Uhuru.

Inverse Compton spectra and the spectrum of Cygnus X-1

THE many X-ray observations of the black hole candidate Cyg X-1 show a variable power-law spectrum (see refs 1-8)

$$\frac{dL}{dE} \simeq (2-6) \times 10^{35} \text{ erg s}^{-1} \text{ keV}^{-1} \left(\frac{E}{10 \text{ keV}} \right)^{-p} \left(\frac{d}{2.5 \text{ kpc}} \right)^2 \quad (1)$$

where d is the distance to the source. During the 'low state', in which the source spends $\sim 90\%$ of its time⁷, $p \simeq 0.5$ and the power-law spectrum extends over $1 \text{ keV} \lesssim E \lesssim 100 \text{ keV}$, with a knee suggested at $\sim 100 \text{ keV}$. During the transient 'high state', which persists for weeks to months, the total luminosity at $\gtrsim 1 \text{ keV}$ increases by a factor 5-10 and the spectrum steepens to $2 \lesssim p \lesssim 4$ over the range $1 \text{ keV} \lesssim E \lesssim 10 \text{ keV}$. A hard excess above this steep slope is visible at $> 10 \text{ keV}$, which contains $\sim 10\%$ of the total energy. In this paper we glean some information about the physics of the source by interpreting this spectrum as being caused by inverse Compton scattering by nonrelativistic electrons at a temperature $T_e \simeq 10^9 \text{ K}$.

A power-law spectrum can arise from two familiar mechanisms when there exists a power-law distribution of ultra-relativistic electrons, namely inverse Compton scattering or synchrotron radiation. Recently, several workers⁸⁻¹¹ have pointed out a third mechanism that produces a power-law spectrum: repeated inverse Compton scattering of initially soft photons by a non-relativistic, thermal electron distribution in a finite cloud, optically thin for emission and absorption. If the soft photons are injected into the cloud with a characteristic photon energy E_s and spectrum dL_s/dE , then the emergent hard spectrum is

$$dL/dE \simeq dL_s/dE (E/E_s)^{-p}; E_s \lesssim E \lesssim kT_e \quad (2)$$

The electron distribution need not be precisely Maxwell-

Boltzmann. The spectral index, p , is determined by the physical properties of the scattering cloud through the relation

$$p = -\frac{3}{2} + \left(\frac{9}{4} + \frac{4}{y} \right)^{1/2} \quad (3)$$

where y is the "Comptonisation parameter", $y \equiv (4kT_e/m_e c^2)$. Max (τ_{es}, τ_{es}^2) and τ_{es} is the electron scattering optical depth of the cloud. For $E \gtrsim kT_e$ (or, because of relativistic effects, $E \simeq 150 \text{ keV}$, whichever is less), the inverse Compton spectrum is no longer given by equation (2) and steepens roughly exponentially. The above spectrum applies as long as $y \lesssim 5$ (unsaturated "Comptonisation"), as long as the cloud acts as a single emitting region, and as long as inverse Compton scattering dominates other cooling mechanisms.

For any total luminosity L dictated, for example, by the rate of gravitational energy release which is powering the X-ray source, and for any luminosity L_s of the soft photon source, the cloud will adjust its y (and p) so that $\int (dL/dE)dE = L$. If the cloud is not emitting enough energy because its spectrum is too soft, the electrons will heat up, y will increase, and the spectrum will get harder; see equations (2) and (3). Changes in either L or L_s can induce a change in y and hence in p .

To interpret the observed spectrum and its variations, let us first extrapolate the observed spectrum to $< 1 \text{ keV}$ and consider that energy E_{RJ} where it intersects the Rayleigh-Jeans portion of a black body spectrum

$$dL_{RJ}/dE \simeq 2 \times 10^{43} \text{ erg s}^{-1} \text{ keV}^{-1} (T_e/10^9 \text{ K}) (E/10 \text{ keV})^2$$

(see Fig. 1). Here we have adopted a radius $\sim 30(GM_{\text{black hole}}/c^2) \simeq 300GM_{\odot}/c^2 \simeq 5 \times 10^7 \text{ cm}$ for the emitting region, typical of most models of Cyg X-1 (a factor of three greater or smaller would make little difference to our results). The intersection is given by

$$\left(\frac{E_{RJ}}{10 \text{ keV}} \right)^{2+p} \simeq 10^{-8} \left(\frac{T_e}{10^9 \text{ K}} \right)^{-1} \left(\frac{d}{2.5 \text{ kpc}} \right)^2 \quad (4)$$

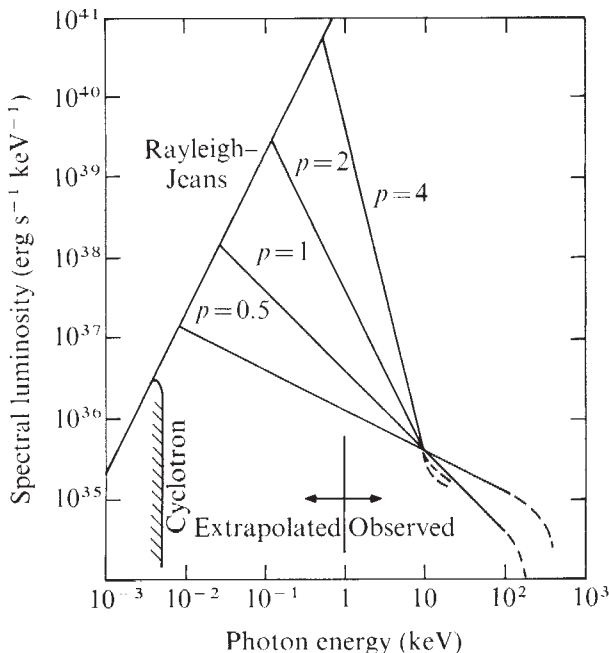
Now, any incoherent soft photon source cannot be more luminous than a black body; therefore we must have $E_s \gtrsim E_{RJ}$ as a strict lower limit on E_s ; in fact, most possible sources (see following discussion) will be optically thick below some characteristic photon energy E_s , so that $E_s \simeq E_{RJ}$.

In the low state, $p \simeq 0.5$ and therefore $y \simeq 2$. If $T_e \simeq 10^9 \text{ K}$, then we learn that $\tau_{es} \simeq 1$ and equation (4) yields $E_{RJ} \simeq 0.01 \text{ keV}$. Thermal cyclotron radiation is likely to be the soft photon source here. The detailed models^{9,12} of Cyg X-1 predict $B \simeq 10^7$ gauss in equipartition, giving $E_{\text{cyclotron}} \simeq 10^{-4} \text{ keV}$, and we calculate (using $\tau_{es} \simeq 1$) that self-absorption occurs up to about the 50th harmonic. Thus $E_s \simeq 0.005 \text{ keV}$, in tolerable agreement with $E_{RJ} \simeq 0.01 \text{ keV}$. Therefore, we suggest that the reason the spectrum of Cyg X-1 is never flatter than $p \simeq 0.5$ is that thermal cyclotron radiation is always present as a minimal soft photon source with $E_s \simeq E_{RJ} \simeq 0.01 \text{ keV}$ and $L_s \simeq 10^{34} \text{ erg s}^{-1}$. Examination of Fig. 1 clearly indicates that additional soft photon sources must lead to steeper spectral slopes.

In the high state, $p \simeq 4$, $y \simeq 0.2$ and almost all of the luminosity emerges near the soft photon input energy E_s . Apparently, a new soft photon source is operating which produces nearly the entire observed luminosity of the source $L_s \simeq L \simeq 10^{38} \text{ erg s}^{-1}$ and which thus requires only a marginal boost (low y) from inverse Compton scattering to balance the rate of gravitational energy release, L . As striking as the observed transitions in state are, we infer an even more spectacular change in the nature of the soft photon source, amounting to a change by a factor of $\sim 10^4$ in L_s .

At $p = 4$, equation (4) gives $E_{RJ} \simeq 0.5 \text{ keV}$ for $T_e \simeq 10^9 \text{ K}$ and even higher E_{RJ} for lower T_e . A plausible candidate for the

Fig. 1 X-ray energy spectrum dL/dE of Cyg X-1. Observations show $dL/dE \propto E^{-p}$, $0.5 \lesssim p \lesssim 4$, for photon energy $E \gtrsim 0.5 \text{ keV}$; these spectra we extrapolate down in E to their intersection with a Rayleigh-Jeans spectrum for $T = 10^9 \text{ K}$. Also shown is a source spectrum for thermal cyclotron radiation.



soft photon source in the high state is a cool, optically-thick accretion disk within the optically thin scattering cloud, since the temperature of a black body of our adopted radius and emitting the observed luminosity of Cyg X-1 would be about 5×10^6 K. At the steepest slope, $p \approx 4$, one should almost certainly see a peak and turnover in the power-law spectrum not far below 1 keV, since a continuation of such a power law much lower than 1 keV would constitute an intensity exceeding the black-body intensity for any reasonable temperature. The excess hard flux seen at $E \gtrsim 10$ keV must originate in a different region of the source or by a different mechanism, according to this view.

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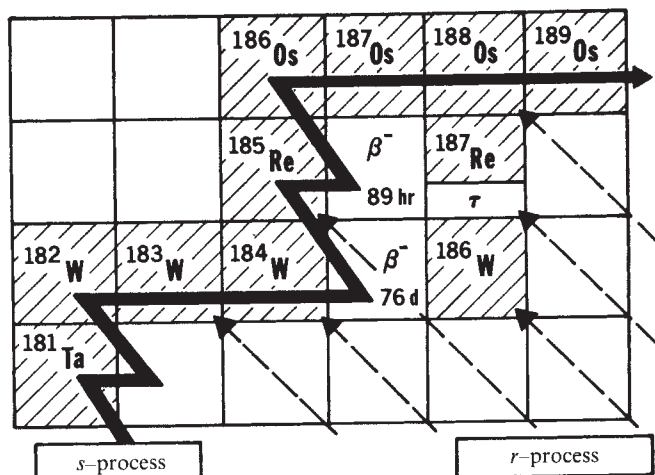
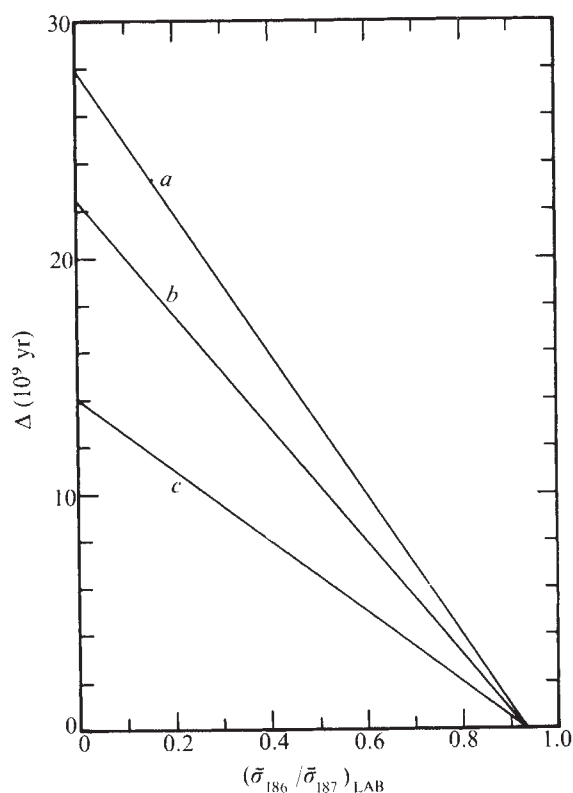


Fig. 1 The *s*-process (heavy line) and *r*-process (dashed line) paths in the vicinity of the Os isotopes. The shaded boxes represent stable nuclei except for ^{187}Re ($\tau_{1/2} = 44 \times 10^9$ yr). ^{186}Os and ^{187}Os are shielded from the *r*-process by ^{186}W and ^{187}Re , respectively.

which has a half-life of $\sim 44 \times 10^9$ yr and which therefore is much longer than estimates of the age of the Universe obtained from the above mentioned dating techniques ($(10-15) \times 10^9$ yr). Figure 1 shows a portion of the chart of the nuclides in the vicinity of the Re and Os isotopes. Both the *s*-process and the *r*-process paths are shown. It can be seen that ^{187}Re is an *r*-process nucleus, while both ^{186}Os and ^{187}Os are shielded from the *r*-process and hence their synthesis initially involves only the *s*-process. If we denote the *s*-process abundances of ^{186}Os and ^{187}Os as N_s^{186} and N_s^{187} and the radiogenic component of ^{187}Os

Fig. 2 The duration of nucleosynthesis Δ , before Solar System condensation as a function of the Maxwellian-averaged laboratory neutron-capture cross section ratio for ^{186}Os and ^{187}Os . The three (of the several possible) models for the supernova rate that are shown (a, uniform; b, exponential and c, sudden) are discussed in the text.



Neutron-capture cross sections for ^{186}Os and ^{187}Os and the age of the Universe

WE have measured the neutron-capture cross sections for ^{186}Os and ^{187}Os in the energy range up to 150 keV, corresponding to stellar temperatures up to $\sim 18 \times 10^8$ K. The knowledge of these cross sections enables us to calibrate the $^{187}\text{Re} \rightarrow ^{187}\text{Os}$ nuclear β -decay clock and thus to make a new radiogenic determination of the age of the Universe.

There are several techniques used by astrophysicists to determine the age of the Universe. These include the use of measurements of the expansion of the universe¹ (Hubble constant) and of observations of stars in globular clusters² as well as nucleosynthetic techniques. All of the techniques involving nucleosynthesis used to date, however, have been based on the theory of the *r*-process for the formation of the elements³. The *r*-process involves neutron capture on a rapid time scale in supernova events. It follows a path off the line of β stability and is responsible for the formation of all of the elements heavier than bismuth. By calculating the production ratios for various *r*-process nuclei such as ^{232}Th , ^{235}U and ^{238}U in supernova events and by measuring the current abundance ratios of the same nuclei, it is possible to arrive at a chronology of supernova events (and hence at the age of the Universe). This is the basis of the U-Th dating technique of Fowler and Hoyle⁴.

In 1964, Clayton⁵ suggested the use of a process which does not depend critically upon a knowledge of the *r*-process production rates. Rather it depends mainly upon the *s*-process, which rests upon a more firm theoretical footing. The *s*-process refers to neutron capture on a much slower time scale than the *r*-process, and it follows essentially the line of β stability. Clayton's suggestion involves the β decay of ^{187}Re to ^{187}Os ,

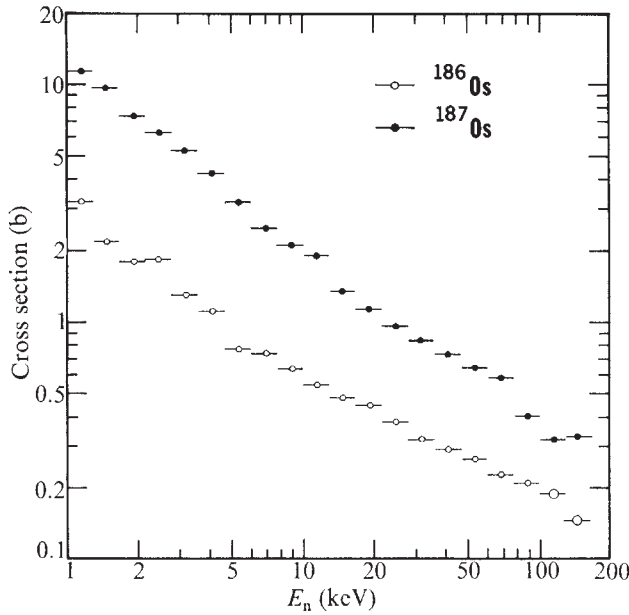


Fig. 3 The neutron-capture cross sections against laboratory neutron energy for ^{186}Os ($\circ$) and ^{187}Os ($\bullet$). The horizontal bar represents the size of the energy bin into which the data have been averaged. (The energy resolution of the actual measurement was much better.) The uncertainty for each data point lies within the plotted symbol and typically is 5% at 30 keV.

resulting from the ^{187}Re β decay as N_{rad}^{187} , then the total abundances can be written as

$$\begin{aligned} N^{186} &= N_s^{186} \\ N^{187} &= N_s^{187} + N_{\text{rad}}^{187} \end{aligned} \quad (1)$$

One of the assumptions of the s -process is that $N\bar{\sigma}$ is constant for adjacent s -process nuclei, where $\bar{\sigma}$ refers to the Maxwellian-averaged neutron-capture cross section for a temperature appropriate to the site of the s -process ($kT \approx 30$ keV). This assumption has been justified by Macklin *et al.*⁶ for the tin, samarium, strontium, and zirconium isotopes. Using this result and equation (1), it is possible to write the ratio of the radiogenic component of ^{187}Os to its parent ^{187}Re abundance N_{Re}^{187} as

$$\frac{N_{\text{rad}}^{187}}{N_{\text{Re}}^{187}} = \frac{(N^{187}/N_{\text{Os}}) - f(\bar{\sigma}_{186}/\bar{\sigma}_{187})_{\text{LAB}}(N^{186}/N_{\text{Os}})}{N_{\text{Re}}^{187}/N_{\text{Re}}^{186}} \left(\frac{N_{\text{Os}}}{N_{\text{Re}}} \right) \quad (2)$$

where N_{Os} and N_{Re} refer to the elemental abundances of Os and Re. The measured laboratory cross-section ratio $(\bar{\sigma}_{186}/\bar{\sigma}_{187})_{\text{LAB}}$ must be multiplied by the factor f since the Os nuclei in a stellar environment exist in excited states as well as the ground state. The most probable value for this factor has been calculated by Woosley and Fowler (personal communication) to be $f = 0.83$. The values used for the other parameters in equation (2) are listed in Table 1.

Clayton⁵ used a simple model to determine the above ratio as a function of the time and duration of nucleosynthesis. He

assumed that r -process nucleosynthesis (which formed ^{187}Re) began at a time Δ before the condensation of the Solar System and decreased exponentially at a rate λ_A . Therefore λ_A is a measure of the supernova rate in the Galaxy. If λ_B denotes the β -decay rate of ^{187}Re , one can express the ratio $N_{\text{rad}}^{187}/N_{\text{Re}}^{187}$ at the time of Solar System condensation, using the Bateman equations for radioactive growth and decay, as

$$\frac{N_{\text{rad}}^{187}}{N_{\text{Re}}^{187}} = \left\{ \frac{\lambda_A - \lambda_B}{\lambda_A} \exp(\lambda_B \Delta) \frac{1 - \exp(-\lambda_A \Delta)}{1 - \exp[-(\lambda_A - \lambda_B) \Delta]} \right\} - 1 \quad (3)$$

The two extreme cases of this model are (1) sudden synthesis (a single supernova event), for which $\lambda_A \rightarrow \infty$ and equation (3) becomes $N_{\text{rad}}^{187}/N_{\text{Re}}^{187} \approx \lambda_B \Delta$, and (2) uniform synthesis (a constant rate of supernova events), for which $\lambda_A \rightarrow 0$ and equation (3) becomes

$$N_{\text{rad}}^{187}/N_{\text{Re}}^{187} \approx \lambda_B \Delta / 2$$

It should be noted that not only does case (1) result in the minimum value for Δ , but this value also is the average value for Δ , irrespective of the rate (or the rate of change) of supernova events, and hence independent of the model used for the time history of the stellar burning process.

If we now plot Δ as a function of $(\bar{\sigma}_{186}/\bar{\sigma}_{187})_{\text{LAB}}$ using equations (2) and (3) we obtain the curves shown in Fig. 2. The two extreme cases mentioned above are plotted along with an intermediate case where the supernova rate is assumed to be exponential in time, with its activity at Solar System condensation equal to $1/e$ of its initial value ($\lambda_A = 1/\Delta$). For the curves in Fig. 2, the β -decay half-life measurement of Herr *et al.*⁷ ($\tau_{1/2} = 44 \times 10^9$ yr) was used for λ_B .

The purpose of our measurement therefore was to determine the ratio of the neutron-capture cross sections for ^{186}Os and ^{187}Os , Maxwellian averaged for $kT = 30$ keV, from which $N_{\text{rad}}^{187}/N_{\text{Re}}^{187}$ can be obtained using equation (2). From this ratio, the duration of nucleosynthesis (Δ), and hence the age of the Universe, can be obtained as discussed above. This measurement was performed using the neutron time-of-flight facility at the Livermore 100-MeV electron linac. Details of the experimental techniques used for these neutron-capture cross-section measurements are given in ref. 8. For the present experiment, the neutron-capture cross sections for ^{186}Os and ^{187}Os were measured over the neutron-energy interval from 2 eV to 150 keV, using a 3.278-g powdered-metal osmium sample enriched to 78% isotopic purity of ^{186}Os and a similar 2.959-g sample enriched to 71% isotopic purity of ^{187}Os . Since the impurities consisted of the other stable Os isotopes, the neutron-capture cross sections for all these isotopes were measured as well. The data were reduced to absolute cross sections by including a ^{165}Ho sample in the measurement and normalising to the measured $^{165}\text{Ho}(n, \gamma)$ cross section⁹. The resulting average neutron-capture cross sections obtained for ^{186}Os and ^{187}Os are shown in Fig. 3. These cross sections were Maxwellian averaged for $kT = 30$ keV, from which the value for $(\bar{\sigma}_{186}/\bar{\sigma}_{187})_{\text{LAB}} = 0.39 \pm 0.03$ was obtained. The uncertainty in this ratio includes systematic as well as statistical uncertainties in the measurement. Finally, the value obtained for $(\bar{\sigma}_{186}/\bar{\sigma}_{187})_{\text{LAB}}$ is only weakly dependent upon the choice of kT near 30 keV.

This measurement of $\bar{\sigma}_{186}/\bar{\sigma}_{187}$ therefore removes the major unknown in equations (2) and (3) that prevented, until now, the determination of the age of the Universe by the $\text{Re} \rightarrow \text{Os}$ clock. From Fig. 2, a value for $\Delta = (12.9 \pm 3) \times 10^9$ yr is obtained using the above value for the cross section ratio for the exponential case. (This 23% uncertainty results from the 8% uncertainty for the present measurement together with the uncertainties in the other quantities in equation (2).) This value can be compared directly with the U-Th dating method⁴, which yielded $\Delta = (7 \pm 2) \times 10^9$ yr using the same exponential description.

Table 1 Values used in equation (2) to compute $N_{\text{rad}}^{187}/N_{\text{Re}}^{187}$

N^{187}/N_{Os}	0.0123 ± 0.0006
N^{186}/N_{Os}	0.0159^*
$N_{\text{Re}}^{187}/N_{\text{Re}}^{186}$	0.65^*
$N_{\text{Os}}/N_{\text{Re}}$	12 ± 1.2

These values are averages of results obtained by geochemical methods, referred to the time of solar system condensation, using $\tau_{1/2} = 44 \times 10^9$ yr from ref. 7.

*The geochemical uncertainties for these quantities are negligible.

Thus there is a substantial discrepancy between the results of the two dating techniques.

It should be pointed out that the $\text{Re} \rightarrow \text{Os}$ dating technique depends strongly on an accurate determination of $\lambda_{\text{Re}} (= 1/\tau_{\text{Re}})$. At the present time, the best two measurements^{7,10} of this half life disagree by 50%. We have chosen to use the measurement of Herr *et al.*⁷ in our determination of Δ . Since the value of τ_{Re} from ref. 10 is much larger than that reported in ref. 7, the discrepancy between the present result for Δ and that obtained from the U-Th method would be increased considerably if the value for τ_{Re} from ref. 10 were used. There are other uncertainties involved in the $\text{Re} \rightarrow \text{Os}$ method, some of which are discussed in detail by Clayton⁵. Another is the possibility that some of the ^{187}Re , and hence some of the radiogenic ^{187}Os , is produced by the *s*-process. These uncertainties involve, however, effects that are either not well understood or of a speculative nature; therefore they will not be discussed further here.

If, then, we assume that the time between the end of nucleosynthesis in the Galaxy and the present time is 4.7×10^9 yr, we obtain the age of the Galaxy to be $\Delta + 4.7 = 17.6$ (in units of 10^9 yr). The time between the beginning of the Universe and the formation of the Galaxy is not known, but current estimates¹¹ give $\sim 2 \times 10^9$ yr. Therefore the $\text{Re} \rightarrow \text{Os}$ dating method, based on the results reported here, indicates that the age of the Universe is $\sim 19.6 \times 10^9$ yr (and the uncertainty in this number probably is no greater than 4×10^9 yr). The present results thus yield an age which is greater than other recent estimates of this age, from the expansion of the Universe $((16.6 \pm 1.7) \times 10^9$ yr, ref. 12), from globular clusters $((13 \pm 3) \times 10^9$ yr), and from the U-Th method $((14 \pm 3) \times 10^9$ yr).

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Gravity signature of fossil plate boundaries in the Canadian Shield

THE Canadian Shield is a mosaic of structural provinces, each with characteristic internal structural trends and styles of folding¹. Boundaries between provinces have been delineated where trends are truncated along unconformities or along orogenic fronts. The validity of this subdivision of the shield has been supported by isotopic dating of orogenies². Thus, the Superior province is the type region for the Kenoran Orogeny (2,560 Myr BP), and the Churchill and Grenville provinces are the type regions for the Hudsonian (1,800 Myr) and Grenvillian (1,000 Myr) orogenies, respectively.

Gravity signatures observed across structural province boundaries in five different areas are remarkably similar (Figs 1 and 2a). We suggest here that they originate from essentially identical crustal structures formed in response to fundamental processes operating during Precambrian episodes of cratonic convergence, collision and suturing. Three geosutures have so far been identified at or near structural province boundaries in

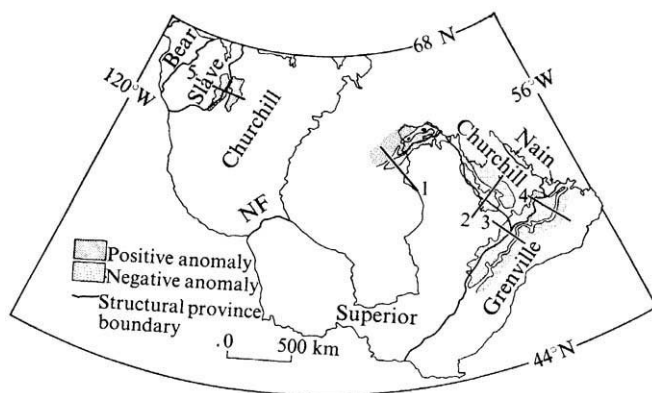
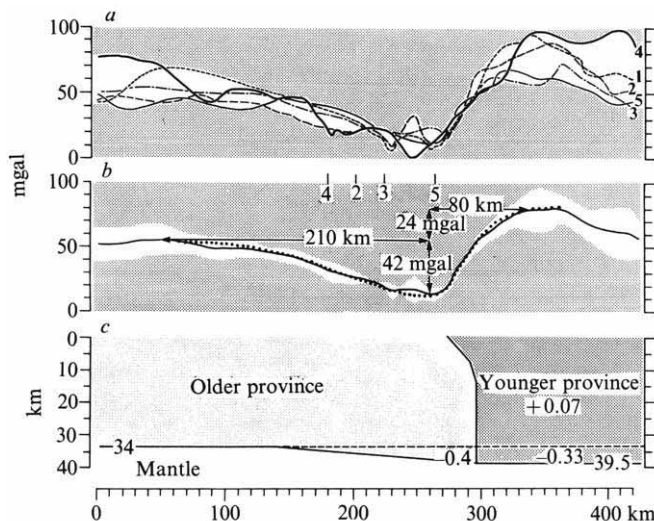


Fig. 1 Bouguer gravity anomalies at five structural province boundaries in the Canadian Shield. Gravity profiles (shown in Fig. 2a): 1, Superior-Churchill boundary (offshore extension of Cape Smith Belt); 2, Superior-Churchill boundary (Labrador Trough); 3, Superior-Grenville boundary (Grenville Front); 4, Churchill-Grenville boundary (Grenville Front); 5, Slave-Churchill boundary (Thelon Front). NF, Nelson Front.

the shield. The suture peripheral to the Superior Craton is the most easily recognised³⁻⁵ because it is characterised by distinctive Aphebian (Lower Proterozoic) geosynclinal and oceanic sedimentary, volcanic, and ultramafic rocks, or their metamorphosed equivalents. The interpretation of these rocks as a suture therefore depends on the juxtaposition of pseudo-ophiolites typical of the plate accretion environment and geosynclinal sediments typical of zones of plate consumption⁴. The suture extends for some 3,200 km from the Manitoba nickel belt (Nelson Front) through the Split Lake-Fox River region of Manitoba and the Hudson Bay Lowlands of northern Ontario to eastern Hudson Bay and thence to the Cape Smith Belt and Labrador Trough of northern Quebec (Fig. 1). Reactivated basement of the Churchill Province is sharply separated by the suture rocks from the non-reactivated Superior Craton in Manitoba, Ungava and Quebec-Labrador^{6,7}. Interpretations of large amplitude Bouguer anomalies associated with the Cape Smith Belt⁸ and Labrador Trough^{7,8} indicate that the cratons

Fig. 2 a, Bouguer gravity anomaly signatures along profiles 1-5 of Fig. 1 (vertical lines numbered 2-5 show positions of corresponding inter-province boundaries²²); b, gravity signature (type anomaly) derived by averaging profiles 1-5. Unshaded envelope is described by standard deviation calculated at 5-km intervals along profiles; dotted curve, gravity effect of type model shown in c. c, Type crustal structure derived from type anomaly. Density contrasts in g cm^{-3} ; depths in km



separated by the suture have different crustal densities and thicknesses.

Interpretations of palaeomagnetic data⁹⁻¹¹ suggest that part of the Grenville Province broke away from the remainder of the shield 1,250 Myr ago, and then rejoined it about 1,000 Myr ago. These events require that a suture also exists within the Grenville Province. Different locations have been suggested for such a suture¹¹⁻¹³. Most recently, Thomas and Tanner¹⁴ have suggested that the suture lies up to 100 km inside the Grenville Province, south of a zone believed to contain reworked rocks of the older adjacent provinces lying to the south of the Grenville Front¹¹. This location is based on the interpretation of gravity anomalies along the front as an edge-effect between juxtaposed crustal blocks of different mean density and thickness^{8,14,15}. The boundary between the blocks is the suture. It is cryptic in the sense that no clear surface indications of a suture have yet been recognised. Petrological investigations indicate, however, that rocks at present at the surface in this region were formed at temperatures of 700–750 °C, and at depths of about 20 km (ref. 12). At these crustal levels, ultramylonites and pseudotachylites are likely to be the only indicators of the line of suturing⁶.

A third suture has been postulated at or near the metamorphic boundary between the Slave and Churchill provinces^{16,17}; it is known as the Thelon Front¹⁸. Belts of cataclastic gneiss and mylonite occur roughly parallel with the front inside the Churchill Province¹⁹. The front is bounded on either side by coextensive negative (Slave) and positive (Churchill) accurate gravity anomalies (Fig. 1) which have also been interpreted as an edge-effect between juxtaposed crustal blocks of different mean density and thickness. According to this interpretation¹⁷ the density discontinuity penetrates the crust and represents a cryptic suture between collided cratons. The gravity anomaly is almost identical to those found at the western extension of the Cape Smith Belt, the Labrador Trough, and the Grenville Front.

Bouguer anomaly profiles numbered 1–5 across these boundaries (Fig. 1) are shown on a common datum in Fig. 2a. Apart from short wavelength anomalies attributable to local geological features, all five profiles are remarkably similar. In each case the gravity profile decreases gradually from a background level over the older province to a minimum near the inter-province boundary zone, and then increases sharply to a broad maximum over the younger province, some 16 to 40 mgal higher than over the older province. The smoothed gravity signature, which we call the type anomaly, derived by averaging profiles 1 to 5, is shown in Fig. 2b within an envelope which varies according to the standard deviation calculated at intervals of 5 km along the profiles. If the anomaly over the older province is regarded as background, the amplitudes of the minimum and the maximum are –42 mgal and 24 mgal, respectively. The average gradients over the older and younger province margins are about 0.2 mgal km⁻¹ and 0.8 mgal km⁻¹, respectively. Near the minimum, the gradient is much steeper (up to 1.8 mgal km⁻¹) over the younger province.

A type crustal model has been derived from the type anomaly; it is constrained in three ways. Seismic results^{20,21} suggest that the crust underlying both the Superior and Slave provinces is about 34 km thick in the vicinity of the profiles. This thickness was assigned to the crust of the older province along with an assumed normal crustal density of 2.84 g cm⁻³. The density of the upper mantle was assumed to be 3.24 g cm⁻³. Keeping these parameters fixed the thickness and density of the younger crustal block were varied until the calculated anomaly matched the type anomaly. The crustal model is shown in Fig. 2c, and the computed anomaly in Fig. 2b. The crustal thickness of the younger province is 39.5 km, and the density is 2.91 g cm⁻³. The density discontinuity dips towards the younger province from a point 13 km from the minimum, to a depth of 14 km at a distance of 35 km from the minimum, where it becomes vertical over the rest of its length. The base of the crust of the older province dips gently towards the contact over a distance

of 160 km. This non-isostatic crust is required to fit the gravity minimum. The blocks are otherwise in approximate isostatic equilibrium.

The model indicates that the younger crustal block is consistently thicker and slightly denser than the older. This consistency is surprising, but all the more surprising because not only do the structures have a wide geographic distribution but they are also of vastly different ages. This suggests that similar processes have operated throughout much of Precambrian time. The density discontinuity of the type model penetrates the whole crust and separates cratons of different density, thickness, age and internal structure; it is interpreted as a vestigial suture between collided continental blocks. The model with slight modifications to crustal density and thickness can be applied to all five boundary zones and may apply to other examples in Canada and elsewhere wherever the gravity signature of the type model is recognised.

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Dating of historical earthquakes by mud profiles of lake-bottom sediments

RECENTLY, Ben-Menahem *et al.*¹ derived tectonic patterns and slip rates in the Jordan Rift Valley by combining a comprehensive study of seismological, historical, geological and morphological data. It was found that the principal seismic activity along the Jordan Valley is due to a left-lateral strike-slip fault, striking 11° east of north and extending for about 50 km from 32°N to 31°30'N along the 35°30' meridian. Its most active part in the past four millennia has been near the monastery of John the Baptist which, according to tradition, is the place where the Israelites crossed the Jordan and where Jesus was baptised. The fault trace and its role in the historical seismicity of the area is shown in Fig. 1. It has an estimated mean rate of activity of 2–3 events per century at magnitudes from 5 to 7 (ref. 1). An aseismic slip rate of at least 3 mm yr⁻¹ is taking place along this fault¹. Here we show how this activity is reflected in an unusual limnological process which takes place at the bottom of the Dead Sea and which can serve as a 'geophysical clock' to date historical events.

The fault's seismicity has been reflected in many historical records, in particular the Bible, in which we find distant echoes of tectonic events as remote as 2000 BC. Lists of earthquakes have been compiled from many and diverse sources (see, for example, refs 2–5), and a new revised list,

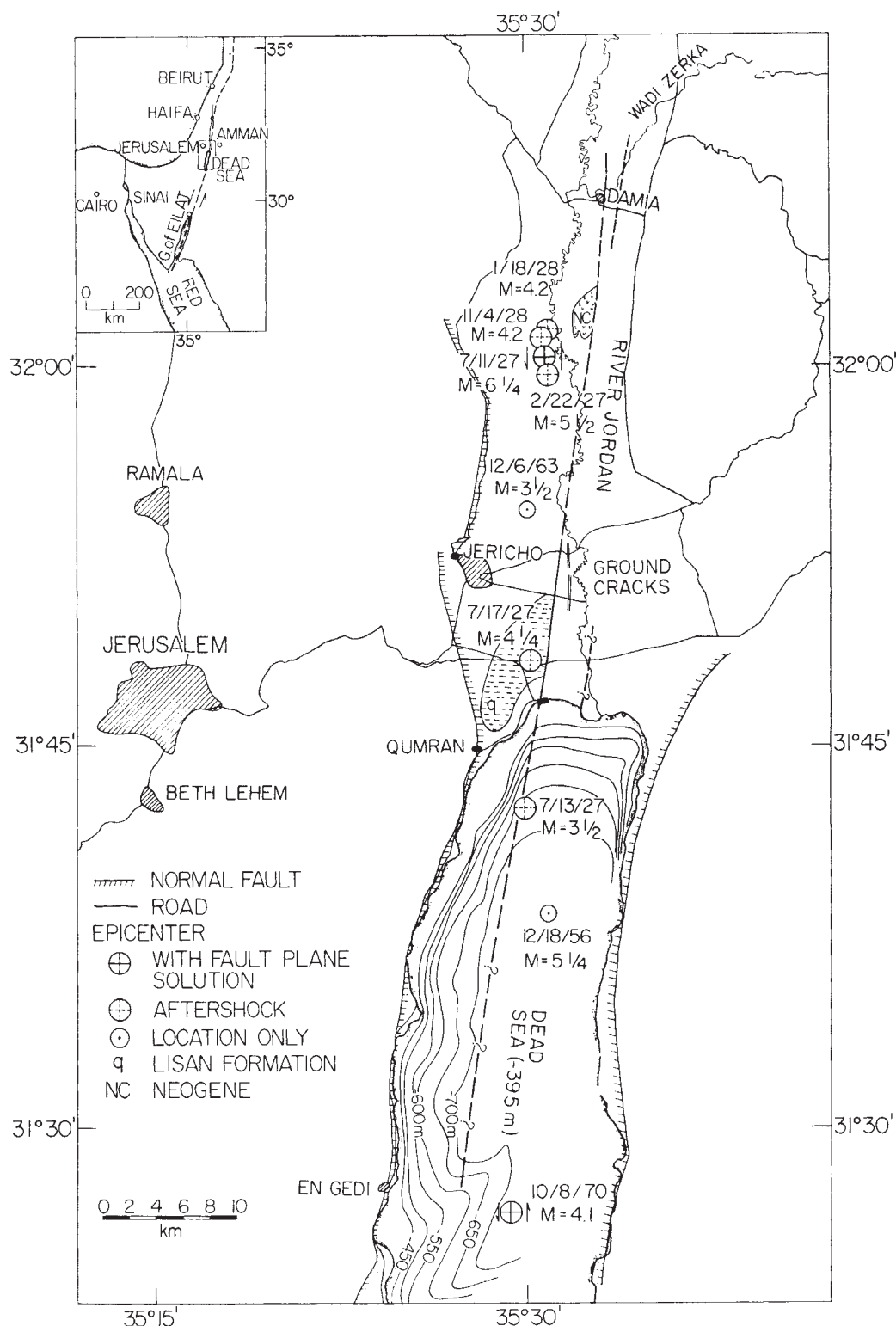


Fig. 1 Map of the Dead Sea fault (see ref. 1).

which is believed to include seismic events above a Richter magnitude 5, is given in ref. 1.

That list, although inaccurate and incomplete in detail, probably includes the major seismic events that have taken place around the Dead Sea fault during the past 4,000 yr. Although many of the epicentres are known with poor accuracy, the list suggests that the number of intermediate and major earthquakes in the area ($M=5-7$) was about 50 in the past 2,000 yr, yielding an average 'quiet' interval of 40 yr. We have used observations of Elazari-Volcani⁶ who obtained a number of profiles of mud samples from depths of 70–330 m at different places in the Dead Sea

during December 1941. At one place about 6 km south-west of the northern shore, a profile 170 cm long was obtained from a depth of 100 m. On dissecting the profile longitudinally, a 'spectrum of layers' of different colours—black, dark-blue, grey brown and white—was revealed (Fig. 2). The zones of sedimentation, distinctly seen, form no seasonal repeat pattern, as do, for example, the annual rings of trees. Similar observations were reported for many other lakes by Perfiliev⁷.

On September 10, 1943, an earthquake was felt along the Jordan Valley. Macroseismic evidence placed the epicentre at the northern tip of the Dead Sea fault, with an estimated



Fig. 2 'Spectrum' of layers of a mud profile of 170 cm long obtained from the bottom of the Dead Sea at depth of 100 m. Section starts at upper end of *a* and ends at the lower end of *e*.

Richter magnitude of 5. About one week before this event, new springs opened up near the shores of the Dead Sea and disgorged white material into the Sea, causing prolonged whitish discoloration for about 5 months. Ashbel⁸ conjectured that the white layers in the core samples of Elazari-Volcani⁶ could have been caused by the sedimentation of that white material. He counted 50 white layers in the total of 2,000 yearly layers seen in the 'spectrum' of Elazari-Volcani⁶. It is therefore tempting to correlate the

time intervals of our chronological list with the length intervals of the white layers in Fig. 2.

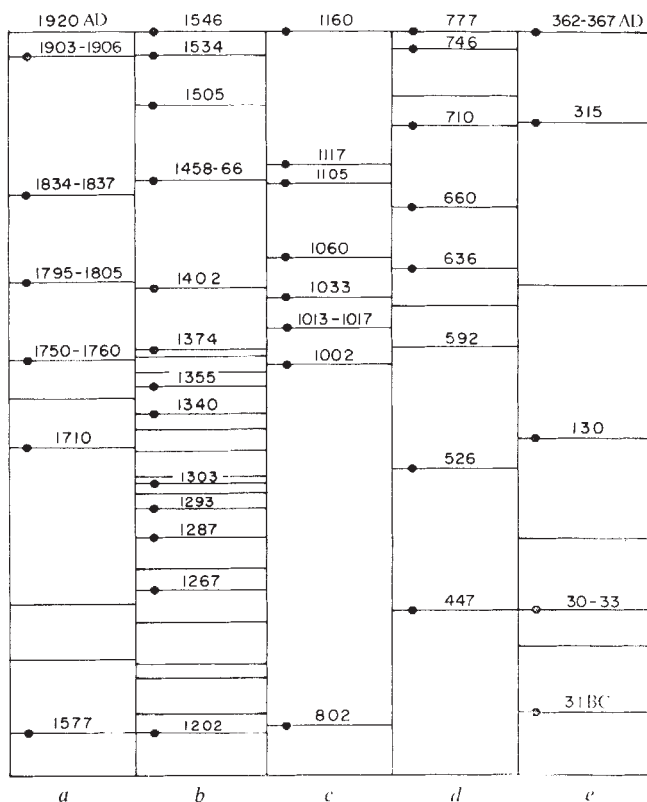
Starting with the conspicuous white layer in the upper part of a column *a* (opposite the numbers 2/3) we associate it with two earthquakes that occurred during this century on the Dead Sea fault (1903, 1906; see ref. 1). The next white layer is in the same column opposite the numbers 7/8. Assuming that the thickness of the layer is proportional to the magnitude, this could correspond to the great shock of May 23, 1834 ($M=6.25$) which is also known to have caused a white discoloration of the Dead Sea. This permits a 'calibration' of the time scale in Fig. 2 (18.5 yr per scale unit). Figure 3 shows the correlation between the white layers and what I believe to be the corresponding seismic events that gave rise to these layers. All in all, 65% of the white layers could be matched to listed earthquakes. There is an uncertainty of about 10 yr in the dating of some layers and there are also white layers for which no corresponding event could be found in the available lists. One should, however, allow for the possibility that some of the listed earthquakes occurred away from the fault, such that the white discoloration was faint, and that the time scale on the mud profile was not always linear.

I suggest, therefore, that there is a possible correlation of times of earthquake occurrence on the Dead Sea fault and the location of the white layers in the mud profile. There is no doubt however that the mud sample represents a period of about 2,000 yr and that the white layers *in toto* were definitely caused by earthquakes (or by accelerated pre-earthquake creep) on the Dead Sea fault during the past two millennia. I therefore predict that deeper mud cores will reveal white layers that were caused by earlier seismic events. We may be even able to see the white signature of the catastrophe of Sodom and Gomorrah.

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Fig. 3 Dating of white layers of Fig. 2 with the aid of a chronological list of earthquakes.



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Ordered powder mixing

UNTIL recently, the only accepted theory of powder mixing was based on a statistical randomisation of non-cohesive particles¹. The introduction of the concept of ordered mixing² enables a logical theory to be established for the mixing of small cohesive particles to a considerable degree of homogeneity. The concept of ordered mixing has attracted considerable support³⁻⁵. A basic principle of ordered mixing is that fine particles will adhere, especially to larger particles. The adhesional forces involved may be electrostatic or surface tensional. For a homogeneous system to be formed, the weight of fine particles adhering to unit surface area of the larger particles should be constant. We report here such a condition in the mixing of a fluid energy milled closely sized fraction (2.6 μm) of salicylic acid with a multi-sized distribution of sucrose crystals, in the ratio 1 : 1,000 by weight.

The powders were mixed in a Revolve-Cube rotating cube mixer for 6 h. The sucrose crystals were then classified by sieving. The collected fractions were each dissolved in 50%

Table 1 Ordered mixing of microfine salicylic acid with coarse sucrose crystals

Mean size fraction sucrose crystals (μm)	Weight of fraction (%)	Weight of salicylic acid/unit weight of sucrose ($\times 10^{-4}$)	Weight of salicylic acid/unit surface area of sucrose ($10^{-7} \text{ g cm}^{-2}$)	Surface area of sucrose coated (%)
462.5	3.34	4.58	5.61	2.04
		4.73	5.78	2.10
		4.52	5.52	2.01
390.0	20.10	7.10	7.31	2.67
		6.62	6.80	2.50
		6.41	6.59	2.41
302.5	52.43	9.05	7.22	2.63
		9.00	7.19	2.62
		8.74	6.98	2.54
215.0	21.02	13.1	7.46	2.72
		13.8	7.87	2.86
		12.9	7.32	2.67
168.0	2.34	17.0	7.39	2.69
		18.6	8.10	2.94
		16.9	7.35	2.67
137.5	0.78	19.3	7.10	2.56
		21.4	7.37	2.84
		19.8	7.20	2.63

ethanol and assayed for salicylic acid content. The results are given in Table 1.

Over the range of particle size, the weight of salicylic acid per unit surface area of sucrose crystals (assuming cubic crystals, as observed microscopically) is constant within the range of experimental error. From the projected area of the fine particles, it is possible to calculate that ~2.5% of the area of the larger sucrose particles is coated with fine particles at this level of mix dilution.

Segregation was not observed during the classification operation, as demonstrated by the lack of material passing through the finest sieve used.

These results demonstrate the possibility of ordered mixing processes for producing highly homogeneous mixtures of powdered materials. The application of this process to a wide number of different industrial uses is anticipated.

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Krishnaswami *et al.*⁵ have found that three manganese nodules from the Pacific basin concentrate considerable activities of ¹⁰Be at their surface (9–68 d.p.m. kg⁻¹). These concentrations are several times higher than those found in pelagic sediments. We report here on a search for ²⁶Al in manganese nodules.

The TECHNO-1 manganese nodule, obtained by a dredge at a depth of 4,020 m at the north of Tuamotu archipelago has a crust ~ 1,000 cm² in area and ~ 3 cm thick. This crust covers a base of phillipsite. Half of the crust (425 cm²) was provided for the present study. The crust was sliced horizontally into four sections: section I (0–2.3 mm), section II (2.3–8 mm), section III (8–14 mm), and section IV (14–23 mm).

For the analysis of ²⁶Al, the nodule material was leached twice in hot, 6 N HCl. After being isolated by centrifugation, the insoluble phases were completely dissolved in HF and the HCl-leached fraction and the HF-dissolved fraction were treated separately for further purification. Aluminium was precipitated with ammonium hydroxide, and iron, manganese and thorium were removed by the dissolution of aluminium hydroxide with NaOH. This purification process was repeated three more times, each time with the addition of a solution of Fe carrier which serves as scavenger for Th. The purified Al was finally converted to Al₂O₃ and counted. In our study, the HCl fraction and the HF fractions were counted together. The chemical yield of Al was 65 ± 5%. The Al content of the nodule was 2%.

²⁶Al decays mostly by the emission of a positron followed by a 1.83-MeV γ ray. The positron thermalises and produces two 0.511-MeV γ rays, enabling the concentration of ²⁶Al to be measured by the coincident detection of a 0.511-MeV γ ray in one counter, and the sum peak of the other two γ rays (total 2.34 MeV) in another. The measurements were carried out with a low-level γ – γ coincidence spectrometer which consists of two semi-cylindrical NaI (Ti) scintillators 30 × 25 cm. The same spectrometer was previously used for our measurements of ²⁶Al in marine sediments⁶. The samples from the manganese nodule were counted for 30,000–60,000 min and the background, for 60,000 min. The background count rate was 0.0137 ± 0.0005 c.p.m. The counting efficiency for ²⁶Al was 5.3 ± 0.3%.

²⁶Al was detected in the first section, where its concentration of 0.29 ± 0.14 d.p.m. kg⁻¹ (a net count rate of 15 ± 7 × 10⁻⁴ c.p.m.) was higher than that found in a Pacific sediment: by our measurement, 0.081 ± 0.046 d.p.m. kg⁻¹ (ref. 6). Therefore the postulated concentration of long lived cosmonuclides in manganese nodule is also confirmed for ²⁶Al.

The enrichment of ²⁶Al in manganese nodule relative to that

Aluminium-26 in a manganese nodule

MANGANESE nodules are generally considered to have accumulated very slowly. Radiometric studies by ²³⁰Th, ²³¹Pa, and ²³⁴U/²³⁸U suggest their growth rates to be a few mm per 10⁶ yr (refs 1–3). This leads to an enigma: how do they escape from burial by associated marine sediments which are accumulating three orders of magnitude faster than the nodules? Though this problem remains open, the postulated slow growth rates might permit manganese nodules to accumulate in a small volume high concentrations of long lived cosmogenic radionuclides ¹⁰Be ($\tau_{1/2}$ = 1.5 Myr) and ²⁶Al ($\tau_{1/2}$ = 0.715 Myr) which were formed through the bombardment of atmospheric constituents by galactic cosmic rays. Indeed, Somayajulu⁴ and

in sediment is more evident when the specific activities (d.p.m. ^{26}Al per kg Al) are compared: 15 ± 7 d.p.m. per kg Al in the manganese nodule and 0.9 ± 0.5 d.p.m. per kg Al in the sediment. This indicates that cosmogenic ^{26}Al precipitates from seawater for preference with oxides, hydroxides and/or authigenic aluminosilicates rather than with detrital silicate minerals.

Measurements of ^{26}Al and ^{10}Be in a Pacific sediment⁶ showed that the $^{26}\text{Al}/^{10}\text{Be}$ ratio in the sediment, 0.109 ± 0.012 , is in good agreement with the theoretical production ratio of 0.013 ± 0.006 as well as with that measured in Greenland ice by McCorkell *et al.*⁷, 0.017 ± 0.008 . From this theoretical ratio and the measured ^{26}Al activity in the nodule, we estimated the ^{10}Be activity in the 0–2.3-mm section of TECHNO-1 to be 22 ± 15 ^{10}Be d.p.m. kg^{-1} . This estimated activity is compatible to the ^{10}Be activities measured by Krishnaswami *et al.* in three manganese nodules: 17, 32, and 89 d.p.m. kg^{-1} (these values are calculated for the 0–2.3-mm section from their measurements of the extrapolated activity at the surface, N_0 , and the depth L at which the activity becomes N_0/e , by using the equation

$$C_x = LN_0 (1 - \exp(-X/L))/X$$

where C_x is the concentration at $O-X$ cm section). The agreement suggests that the $^{26}\text{Al}/^{10}\text{Be}$ ratio in manganese nodules is of the same order as that in sediments, which is expected from the similarity of the geochemical behaviours of the two nuclides in seawaters.

If we assume that the ^{26}Al content decreases exponentially as a function of depth in the TECHNO-1 manganese nodule and that the decrease of ^{26}Al arises from the postulated slow growth rate of nodule material (1–5 mm per 10^6 yr), then it is possible to estimate the ^{26}Al content in the 2.3–23-mm section from the measured content of ^{26}Al in the 0–2.3-mm section (0.29 ± 0.14 d.p.m. kg^{-1}). Concentrations of 0.004 ± 0.002 , 0.06 ± 0.03 , and 0.11 ± 0.05 d.p.m. kg^{-1} are calculated for growth rates of 1, 5 and 10 mm per 10^6 yr respectively. The observed net count rate of $1(\pm 9) \times 10^{-4}$ c.p.m. gives 0.003 ± 0.024 d.p.m. kg^{-1} . The upper limit of the observations for the 2.3–23-mm section, 0.03 d.p.m. kg^{-1} , is therefore compatible with growth rates of 1–5 mm per 10^6 yr.

As a solution to the enigma of the non-burial of manganese nodules by sediments, Arrhenius⁸ and Lalou and Bricet⁹ hypothesised that the radionuclides of interest were not incorporated in the nodule matrix during its growth, but were adsorbed later. The present results for ^{26}Al are not necessarily incompatible with such a hypothesis, because most of the ^{26}Al is found only in the first few millimetres section, but the precision of the present measurements limits further discussions.

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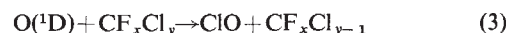
Reactions of $\text{O}(^2\text{D}_2)$ with chlorofluoromethanes and CCl_4

UPPER atmosphere reactions involving chlorofluoromethanes are of considerable current interest^{1–6}. It has been suggested that the diffusion of chlorofluoromethanes, from the troposphere into the stratosphere, where photochemical and reaction processes produce free chlorine atoms, could lead eventually to a depletion of ozone in the upper atmosphere^{1,2}, by way of the ClO_x cycle



The work presented here was directed towards obtaining a more detailed understanding of the primary and secondary processes which take place in the photochemically initiated reaction between O_3 and the chlorofluoromethanes.

The reaction of $\text{O}(^2\text{D}_2)$ with Freons 11–13 (CFCl_3 , CF_2Cl_2 and CF_3Cl) is known to give rise to ClO radicals⁸



and thus provides a means of direct entry into the ClO_x cycle. A number of other channels may, however, contribute to the overall rate of removal of $\text{O}(^2\text{D})$, including quenching to the ground state, $\text{O}(^3\text{P})$. Little is known about the importance of these other channels, or of the subsequent reactions of the $\text{CF}_x\text{Cl}_{y-1}$ radicals which can in some cases give rise to further release of Cl atoms. There is thus a clear need for laboratory based studies to establish the mechanism and kinetics of such reactions.

By using flash photolysis coupled to a fast-flow system with nozzle beam mass spectrometric sampling (Fig. 1), we have obtained direct evidence for a number of transient species which are produced in these photochemical systems. The formation of ClO by reaction (3) is confirmed and the first direct evidence for a competing channel leading to elimination of a halogen molecule (reaction (4) below) is presented. Secondary production of ClO following reaction between CCl_3 and O_3 is also reported.

$\text{O}(^2\text{D}_2)$ atoms were produced by flash photolysing O_3 in the Hartley continuum (200–300 nm). The flash lamp was constructed of quartz and was operated with a continuous flow of argon at a pressure of $\sim 100 \text{ N m}^{-2}$. Flash energies of 125–250 J (10 μF at 5–7 kV) were found to be adequate when signal averaging was used: the half life of the flash at 260 nm was ~ 1 ms. Reagents were mixed just before entry into the flow tube (typically, flow rates of 10 m s^{-1} , with total pressures in the range 300–400 N m^{-2} and O_3 : halocarbon: He = 1 : 10 : 40 were used). He + O_3 mixtures were prepared by diverting part of the He flow through a trap containing O_3 adsorbed on silica gel at 195 K. Ozone was prepared by the method of Clough and Thrush⁷ and the concentration in the flow system monitored optically by absorption at 260 nm; pressures in the range 1–40 N m^{-2} could be conveniently monitored with a path length of 10 cm.

Products of the primary and secondary radical reactions were monitored as a function of time with the mass spectrometer (Extranuclear quadrupole) set to sample a preselected mass number. The analogue output from the mass spectrometer was then digitised and stored in a 200-channel signal averager (Datalab, DL102A); in most cases it was necessary to average the results of sixteen experiments before adequate signal-to-noise ratios were obtained. Little electronic interference from the flash was observed, but some variation with mass number was found and was thus checked for all experimental runs.

Helium (99.9%) and Freons 11, 12 and 13 (Matheson) were taken directly from cylinders. Carbon tetrachloride vapour (laboratory grade) was obtained by pumping on the liquid, and

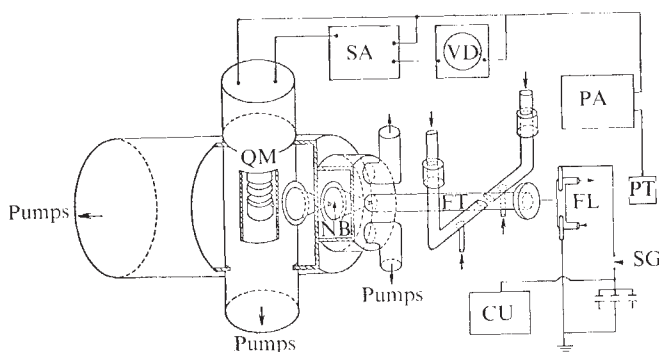


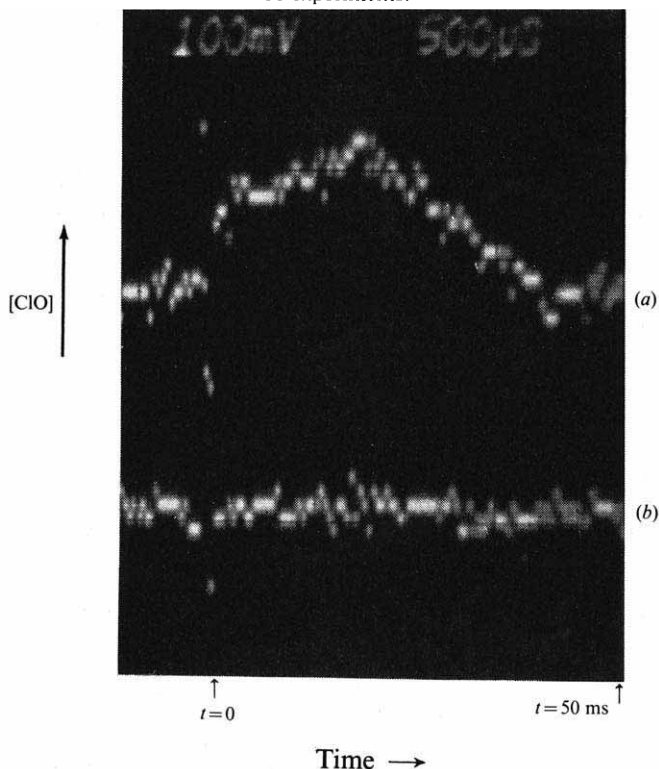
Fig. 1 Schematic representation of the flow tube flash unit and nozzle beam mass spectrometric sampling system: FT, flow tube; NB, nozzle beam sampling system; QM, quadrupole mass spectrometer; FL, flash lamp; CU, charging unit; SG, spark gap; PT, photo-trigger; PA, pulse amplifier; VD, visual display; SA, signal averager.

the flow regulated with a needle valve. The purity of all reagents was checked before an experimental run by direct sampling with the mass spectrometer.

The extent of photolysis for pure ozone was generally in the range 3–10% depending on the flash energies used. In the presence of the chlorofluoromethanes the extent of O_3 decomposition was seen to increase significantly as a result of secondary reactions. An increase by a factor of 3–8 was observed using Freon 11–13: ozone ratios around 5. The effect was less marked for CCl_4 .

Formation of ClO was observed ($m/e = 51$) following the photolysis of O_3 in the presence of Freons 11–13 and CCl_4 (see Fig. 2), supporting previous observations using ultraviolet absorption spectroscopy⁶. No signal at $m/e = 51$ was observed when O_3 was excluded from the flow mixtures under otherwise

Fig. 2 Formation and decay of ClO following the photolysis of O_3 in the presence of CCl_4 . *a*, Ozone, CCl_4 and He mixture: $P_{O_3} = 0.05$ mmHg, $P_{CCl_4} = 0.35$ mmHg, $P_{He} = 2.15$ mmHg. *b*, CCl_4 and He mixture: $P_{CCl_4} = 0.35$ mmHg, $P_{He} = 2.15$ mmHg. Mass spectrometer set to sample $m/e = 51$ (ClO^+), resolution set at 4.25; flow velocity = 10 m s⁻¹; traces are the result of averaging 16 experiments.



identical conditions. The yield of ClO was found to increase with increasing partial pressure of O_3 (over the accessible range 5–13 N m⁻²), as expected from the increase in $O(^1D)$ production. The yield of ClO also increased with the partial pressure of CF_3Cl , however in the presence of CF_2Cl_2 , which is known to give the highest secondary yield of ClO under the conditions used (H. M. Gillespie and, R. J. D., unpublished results), the rate of decay of ClO increased with partial pressure of CF_2Cl_2 (over the range 40–80 N m⁻²). As ClO is unreactive with closed shell species, this indicates that the concentration of free radicals or oxygen atoms increases as the partial pressure of CF_2Cl_2 is increased. This could be caused by a little photolysis of CF_2Cl_2 and will be examined in more detail in future work. The greatest yields of ClO were observed with CCl_4 (Fig. 2).

Other products observed immediately following photolysis of mixtures containing O_3 , were FCl, CFCIO (both from $CFCI_3$) and CF_2O from CF_2Cl_2 . Small yields of CCl_2 and $CFCI$ were also observed from direct photolysis of $CFCI_3$ and CF_2Cl_2 respectively. As these transient species have the same charge-mass ratio at CFCIO and CF_2O , a small correction to yields of the latter species was made. A search was also made for the FO radical, however the large background signal at $m/e = 35$ (Cl^+) made measurements at this mass number very insensitive and no firm evidence for the presence of this radical was obtained.

The present work provides further direct evidence for reaction (3) and the first direct evidence for the channel



(secondary formation of FCl through atom recombination would be too slow to account for our observations). Pitts *et al.*⁴ have observed CF_2O and CFCIO as end products from Freons 12 and 11 respectively and have suggested that reactions similar to (4) were responsible, but secondary reactions can also lead to these end products. Further work to establish the absolute sensitivity of our system for species such as FCl will allow the importance of channel (4) to be assessed quantitatively.

The large yield of ClO from CCl_4 and the continued growth of this radical after the flash (Fig. 2) provides evidence that the CCl_3 radical, formed in the primary reaction between $O(^1D)$ and CCl_4 , reacts further with O_3 , yielding ClO. Little is known about the mechanism of the secondary reactions involving CCl_3 or CF_xCl_{3-x} radicals with O_3 or O_2 , but our work indicates that the $CFCI_2$ radical gives rise to CFCIO, possibly by the reactions



where $*$ denotes vibration excitation, with the chlorine atom reacting further with O_3 to yield ClO.

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Energy analysis of wave-power and wind-power systems

THERE have been several recent suggestions that wave-power systems could provide a substantial part of Britain's energy requirements. The development of such systems presents, however, some formidable problems. One which has been largely overlooked concerns the energy investment required in their construction, compared with the predicted rate of energy return. The calculations which follow suggest that the energy recovery period may be many years. Similar calculations for wind-power systems, whose characteristics are known with greater precision, indicate a very much shorter energy recovery period. Moreover, though the wind is often regarded as a diffuse source of power, it is shown to offer a power density comparable with that assumed for wave-power systems.

Since no large scale wave-power system has yet been constructed, it is not possible to determine precise energy costs. There are, however, enough data available for one proposed system, the Salter rocking float¹⁻³, to provide a reasonable estimate. Salter¹ has shown in laboratory tests that his rocking float is a fairly efficient absorber of wave power and reports that at Station India in the North Atlantic the annual average power in the waves is 77 kW m^{-1} . He therefore suggests that "a few hundred kilometres of installation could meet the total present electrical requirements of the UK". Swift-Hook *et al.*² consider the efficiency of the rocking float for waves of various periods and state that a float 50 m in diameter tuned to a wave period of 10 s should theoretically absorb at least 50% of the incident wave power for wave periods in the range 7–17 s. Measured efficiencies are somewhat lower than theoretical predictions, but an overall average efficiency of 50% for a large scale rocking float may be feasible, and this would give a power output of 38.5 kW m^{-1} for a location comparable with Station India. A rocking float 50 m in diameter would have a displacement of $\sim 2,000$ tonnes (t) per metre length, but if one allows for the connecting structure between floats and the addition of a balancing float to counteract the torque produced by wave action, as suggested by Denton *et al.*³, the displacement will be appreciably higher. Connected arrays of such rocking floats several hundred metres long have been proposed, giving a floating structure comparable in size to modern supertankers. The steel structure of such tankers accounts for about one quarter of their total displacement. If such a ratio can be maintained for wave-power systems, which are appreciably more complex mechanically, the power output of 38.5 kW m^{-1} would require 500 t per m of steel structure, that is the expected power output would be 77 W per t of steel. The energy required to produce 1 t of steel is 38.7 GJ (ref. 4) and it would therefore be 16 yr before the energy output from this wave-power system repaid the energy investment in its construction. Bearing in mind the severity of the working environment, this period is probably comparable with the lifetime of the structure.

The power output per unit displacement may be increased by reducing the diameter of the rocking float system. Smaller floats are, however, less efficient, and marine experience indicates that the ratio of structure weight to total displacement increases as the draught decreases. On balance it seems probable that reduced draught can give some reduction in the energy recovery period, but it is difficult at this time to quantify the improvement that is possible. Moreover, the energy recovery period of 16 yr calculated for the 50-m rocking float is based on assumptions which are somewhat optimistic. For example, Station India is 700 km offshore and well outside the continental

shelf. As Denton *et al.*³ point out, the cost of bringing electricity ashore by underground cable would be prohibitive if the wave-power systems were > 100 km offshore. Locations closer to the shore are therefore necessary and the power in the waves will be attenuated as the waves travel over relatively shallow water. The degree of attenuation will depend on the chosen location, but Salter¹ provides an example of the magnitude of this effect when he quotes the power in the waves at Seven Stones, off Lands End. Here the annual average wave power is 26 kW m^{-1} , one third that of Station India. There will, in any location, be a further loss of efficiency and power output because of the lack of alignment between the axis of the wave-power system and the incident waves. Allowing for all these factors it seems very doubtful whether the energy recovery period for a rocking float system can be reduced to much less than the estimated 16 yr. Other schemes for extracting power from the waves, such as the hinged float system⁵, may possibly have a shorter energy recovery period, but there are insufficient data at present in the literature to provide a reasonable estimate of this.

How do wind-power systems compare with proposed wave-power systems? Over Great Britain as a whole the average power in the wind is at least an order of magnitude greater than the average electricity demand of 30 GW. The wind is usually considered to be a diffuse source of power but in good coastal locations the power flux is comparable with the range quoted for wave-power systems. Wind data⁶ for Stornoway, measured at 12 m above ground level (37 m above mean sea level) indicates a year round average wind speed of 7.4 m s^{-1} and an average power flux of 606 W m^{-2} . Up to a height of 50 m, which is comparable with the draught of proposed wave-power systems, this gives an annual average power flux of 30.3 kW per metre length of wind-power installation. To the east of Britain at Bell Rock, which is 20 km offshore and near the Firth of Tay, the corresponding annual average power flux is 32.9 kW m^{-1} . Modern windmills have a peak efficiency of $\sim 45\%$ but at high wind speeds the efficiency decreases. Over the complete operating range of wind speeds an average efficiency of 30% can be expected. It has been suggested⁷ that 10,000 windmills, each having a rated power output of 1 MW, could produce nearly 10% of our present electricity requirements and save 4.6×10^6 t oil per year. A 1-MW windmill of conventional design would have a diameter of ~ 50 m, and past experience⁸ in the construction of windmills of this size and rating indicates that the average power output would be $\sim 1,090 \text{ W}$ per t of steel. This gives an energy recovery period of ~ 1.1 yr. Modern vertical axis windmills, such as that developed in recent years by Rangi, South and Templin^{9,10}, promise to give a significantly greater output per unit weight and should reduce the energy recovery period for windmills to a few months.

In conclusion, although wave-power systems apparently have the potential to provide a substantial proportion of Britain's energy, the energy investment in their construction seems large by comparison with their rate of energy return. Wind-power systems, however, have a known and very much shorter energy recovery period, and their use on a moderately large scale could make a real contribution towards meeting Britain's energy requirements.

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Dinosaurs, endothermy and blood pressure

THE recent resurgence of speculation about dinosaur physiology and behaviour has resulted in two notable and debatable interpretations. Bakker¹ summarises evidence of several investigators and concludes that dinosaurs were endothermic. Coombs², among others, suggests that the sauropod dinosaurs were mainly terrestrial, only occasionally entering shallow water. These hypotheses might well be viewed in the light of one line of evidence concerning the relationship between height of animals and hydrostatic pressures in the vascular system. Size alone can provide important insights into dinosaur cardiovascular performance which necessarily relates to the questions of endothermy and habitat. This report shows firstly, that high arterial blood pressures in large dinosaurs are consistent with the proposal that they were endothermic. Secondly, I suggest that if the long-necked sauropods were aquatic, they thereby avoided tremendous hydrostatic stresses on the cardiovascular system.

High tissue metabolism in endotherms demands high blood pressure and rapid flow in the systemic circuit to provide adequate exchange of metabolites. Blood flow must be equally rapid in the pulmonary circuit for efficient gas exchange, but the pulmonary blood pressure must be comparatively low to prevent either fluid filtration through the gas exchange surface³ or energy waste inherent in excessive precapillary resistance. The evolutionary trend among vertebrates proceeds from the fishes which perfuse the gas exchanged at higher pressures than the other tissues, through amphibians and reptiles with roughly comparable pressures in two parallel circuits, to endotherms with high systemic and low pulmonary pressures⁴. A four-chambered heart and complete anatomical separation of the circuits seem to be required to maintain the large pressure difference between the circuits in endotherms.

Dinosaurs must have had systemic blood pressures high enough to move the blood from the heart to the head. The terrestrial bipedal ornithopods and theropods, for example, must have had central systemic blood pressures equal to at least the hydrostatic pressure of the blood column above the heart plus the head perfusion pressure. The vertical distances between the heart (centre of anterior body cavity) and the head in several large dinosaurs (Table 1) indicate that central blood pressures characteristic of living reptiles (about 20–70 mmHg)⁵ could not even prevent collapse of the vessels in the head of the large dinosaurs much less provide adequate perfusion pressures. Because it is reasonable to assume that pulmonary blood pressure in dinosaurs was limited by the same constraints as in living air breathers, it is likely that at least these large dinosaurs had four-chambered hearts capable of producing large differences in pulmonary and systemic pressure.

Bakker⁶ noted that, among all living vertebrates, only endotherms include animals with “fully erect gait” and he therefore suggested that dinosaurs were endothermic. By “erect”, however, he meant somewhat more than simply a large heart–head distance and he was criticised for not showing the physiological basis for this correlation between gait and endothermy^{7,8}. The relationship may be that both endothermy and high blood columns require high central systemic blood pressure. Thus, sustained high blood pressure inferred from the large vertical distance between the heart and the head in some dinosaurs supports (but does not prove) the proposition that dinosaurs were endotherms. Perhaps the advent of endothermy with its concurrent development of high systemic perfusion pressure enabled dinosaurs to become large and erect. The

endotherms are the only living terrestrial vertebrates having heads more than about 50 cm above the heart. (Some snakes have long heart–head distances but they are subject to fainting if held in a vertical head-up position⁹.) Consistently, short vertical heart–head distances characterise primitive reptiles which are thought to be ectothermic on the grounds of bone histology (see ref. 11 for literature) and predator–prey ratios in fossil bone deposits¹⁰.

The sauropods present another interesting haemodynamic problem because of the extreme length of their necks. A specimen of *Brachiosaurus* in the Berlin Museum, for example, showed a vertical blood column of about 6.5 m above the heart; this would require a ventricular pressure of over 500 mmHg just to support the column¹². The hearts of other large sauropods also must have had to develop high pressures if these dinosaurs held the head continuously erect and were terrestrial (Table 1). On the other hand, if the sauropods were aquatic, the blood pressure would be more or less equalised by the external hydrostatic pressure, thus placing less strain on the heart muscle.

It can be shown that vertebrate cardiac muscle operates within a limited range of ventricular wall stress. When the stress increases owing to pressure, the myocardium thickens, tending to normalise the stress. Not only does this adaptation occur within individual hearts, it also appears in phylogenetic comparisons. Both mean blood pressure and heart weight are generally 30–40% higher in birds than in mammals^{5,13} for example. Giraffes, which have the highest blood pressure among mammals¹⁴, are said to have hypertrophied hearts¹⁵.

To estimate the degree of hypertrophy in sauropod hearts, I calculated the ventricular wall stresses in ten species of birds and mammals for which appropriate data are available (mouse, rat, dog, man, horse, cow, hen, turkey, and two ducks). The spherical model used is not precise but it is useful in a comparative way. According to the model, the mean stress averages about 1.0×10^5 dyne cm^{-2} ($\pm 0.3 \times 10^5$, the 99% confidence interval) in the species examined. If the heart of a 50-tonne sauropod was the same weight as that of a similarly sized whale (~ 200 kg)¹⁶, it could have sustained pressures within the living vertebrate range. Under the assumptions that it operated within the range of stress indicated above, and it had to withstand pressures greater than 500 mmHg, it is evident that the heart would have been at least eight times heavier than the whale's heart but with the same internal ventricular volume. The spherical model increasingly underestimates stress in real hearts as the wall thickness: ventricular volume ratio increases with higher blood pressure¹⁷. Therefore the estimate of a 1.6-tonne heart is conservative. Such a thick-walled ventricle would be mechanically inefficient and its rate of oxygen consumption would be high. These potential disadvantages of extremely high blood pressure, as well as others listed by Hohnke¹², provide evidence favouring aquatic habits in sauropods.

Table 1 Estimated vertical distances between the head and heart in some large dinosaurs* and the associated hydrostatic pressure of the blood column

	Vertical distance (cm)	Hydrostatic pressure (mmHg)
Ornithopoda		
<i>Hadrosaurus</i>	100–150	77–116
<i>Anatosaurus</i>	200	155
Theropoda		
<i>Ceratosaurus</i>	90–150	70–116
<i>Antrodemus</i>	100	77
<i>Tyrannosaurus</i>	200–240	155–185
Sauropoda		
<i>Apatosaurus</i>	201	156
<i>Brachiosaurus</i>	655	508
<i>Diplodocus</i>	650	502
<i>Camarasaurus</i>	550	425

* Estimates from refs 12, 18 and 19.

Nevertheless, the possibilities remain that the sauropods achieved terrestrial life by possessing extremely hypertrophied hearts or an unusually strong myocardium. Alternatively, they simply might not have raised their heads high, or if they did, they endured a temporary cessation of blood flow to the brain.

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Acetylene reduction by legume root nodule protoplasts

NITROGEN-FIXING protoplasts are of obvious importance in physiological studies of the assimilation of atmospheric nitrogen; in investigations of nodule development; in cloning trials for experimental and agricultural purposes¹; and in somatic cell hybridisation^{2–5}. Production of such protoplasts has proved elusive, however. Davey *et al.*⁶ introduced a technique for the isolation of protoplasts from nodules. Protoplasts prepared by their procedure were incapable of nitrogenase (EC 1.7.99.2)-dependent acetylene reduction⁷, presumably as a result of the extensive loss of leghaemoglobin during the isolation process⁷. By modifying the original procedure⁶, we have obtained naked protoplasts which reduce acetylene after a lag period of about 2 d, and continue to do so for at least 11 d.

Protoplasts were isolated in a medium containing the following: 1% (w/v) Rhozyme HP 150 (Rohm and Haas); 0.5% (w/v) pectin lyase (EC 4.2.2.3 (ref. 8), Kikkoman Shoyu, Chiba-ken, Japan); 1% (w/v) cellulase (EC 3.2.1.4) (Onozuka R-10, Kinki Yakult Co., Tokyo); 1% (w/v) bovine serum albumin (Sigma) and a wash medium consisting of 0.6 M sorbitol; 0.05 M HEPES; 0.3% (w/v) potassium dextran sulphate (to inhibit protoplast fusion)⁹; 0.2 mM KH₂PO₄; 0.1 mM MgSO₄; 0.1 mM CaCl₂; 1.0 μM KI; 0.1 μM CuSO₄; with or without 1 mM KNO₃. The pH of the entire medium was adjusted to 5.8. Protoplasts were prepared from freshly harvested nodules of garden grown *Vigna unguiculata* (L.) Walp plants as previously described⁷. After collection, cells were separated from contaminating rhizobia by decantation in sterile wash medium. Settling of protoplasts was aided by gentle centrifugation (10g for 1 min), fresh wash medium was added and the process was repeated 10–15 times to give a final dilution of any extraneous rhizobia of 10¹⁰–10¹⁵ times. The protoplasts (about 5 × 10⁸ per ml) were incubated in 1.5 ml of wash medium at 25 °C, and acetylene reduction was assayed as described elsewhere¹⁰.

Protoplasts obtained in this manner were intact as judged by light microscopy, possessed most of the original leghaemoglobin content⁷, and began to reduce substantial amounts of acetylene

after a lag period of between 24 and 48 h (Fig. 1a). Further evidence for the structural integrity of the protoplasts was deduced from the observation that when they were plated out on agar suitable for the growth of soyabean callus¹¹, rhizobial colonies were not formed, yet rhizobia from within the protoplasts (UMKL 76) were capable of good growth on this medium when cultured separately. The extent of the lag period could be reduced by removing NO₃[−] from the wash medium used for incubation, but the presence of NO₃[−] seemed to be essential during protoplast isolation. Repression of nitrogenase activity by NO₃[−] is well known¹², and it is possible that NO₃[−] levels must decrease to non-inhibitory concentrations before acetylene can be reduced.

Various substances were added to the incubation medium to test their effect on acetylene reduction. For example, 20 μmol of ATP stimulated it about sixfold (Fig. 1b). Other nucleotides had a similar effect. Similarly, phospho(enol)pyruvate and succinate enhanced acetylene-reduction activity, and the effect in the early stages of treatment was even greater than with ATP (Table 1). Addition of *V. unguiculata* leghaemoglobin to the medium (10–20 μmol) completely inhibited development of acetylene reduction activity. Most of these effects are similar to those seen in cell-free extracts of nitrogenase, and with nitrogenase in bacteroid preparations.

Holsten *et al.*¹³ demonstrated that rhizobia could enter cultured soyabean callus cells. Under the microscope, the rhizobia could be seen within vesicles inside the host cells, and

Fig. 1 a, Rate of acetylene reduction by protoplasts isolated from legume root nodules. Yields were within the range 30–50 mg dry weight of protoplasts extracted from 1 g fresh weight of nodules. Acetylene reduction was assayed in serum bottles of 29.5 ml capacity as described elsewhere¹⁰. Endogenous ethylene production was negligible (<0.01 nmol at the end of the experiment). Each point is the mean ± 1 s.d. of four replicates. **b**, Rate of acetylene reduction by protoplasts incubated with various amounts of ATP (amounts shown in μmol). Protoplasts were isolated from 1 g fresh weight of nodules and yielded 30–50 mg dry weight of material. ▲, +20 μmol; ■, +10 μmol; ○, +5 μmol; ●, control.

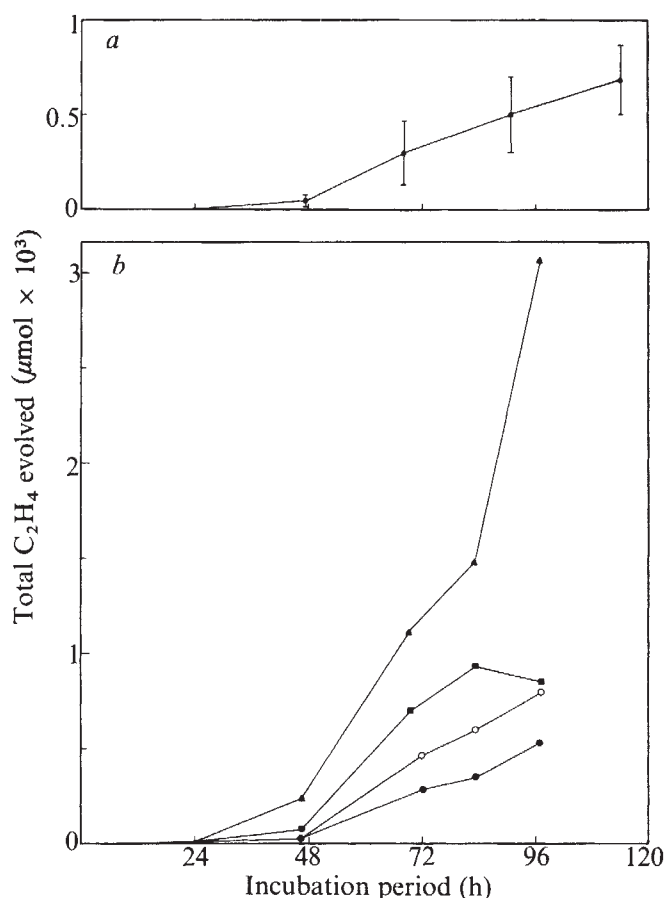


Table 1 Effect of phospho(enol)pyruvate and succinate on acetylene reduction by root nodule protoplasts of *V. unguiculata*

Age of protoplasts (d)	Acetylene reduction (nmol C ₂ H ₄ /mg dry weight/h)		
	Protoplasts	Protoplasts + phospho(enol)pyruvate (40 µmol)	Protoplasts + succinate (40 µmol)
3	0.10 ± 0.01	0.52 ± 0.06	0.17 ± 0.01
4	0.11 ± 0.01	1.98 ± 0.20	1.44 ± 0.09
5	0.09 ± 0.03	3.28 ± 0.91	2.59 ± 0.62
6	1.08 ± 0.04	6.14 ± 0.80	6.20 ± 1.22
7	2.13 ± 0.15	9.42 ± 1.72	6.87 ± 1.70
8	2.04 ± 0.36	8.05 ± 2.04	7.21 ± 1.79
9	3.76 ± 0.32	11.06 ± 3.17	9.25 ± 1.80
11	3.45 ± 0.81	12.57 ± 2.73	13.09 ± 2.46

Each value is the mean (±1 s.d.) of three replicates. Protoplast dry weights were determined at the end of the experiment after oven drying at 80 °C until constant weight.

in this condition reduced acetylene to ethylene. Later work demonstrated acetylene reduction by rhizobia in the presence of plant callus cultures¹⁴⁻¹⁶, although there was no direct contact between bacteria and plant. Then in late 1975 many authors showed that the rhizobia themselves could fix nitrogen in the absence of legumes¹⁷⁻²¹. For these reasons it was imperative to show that the acetylene reduction reported above occurs within the plant membrane of the isolated protoplasts. As discussed, the protoplasts did not release rhizobia when cultured on an agar medium. Also, protoplasts which were actively reducing acetylene could be filtered on to nylon mesh (pore size 40 µm), washed extensively, and still retain the nitrogenase activity even in the semi-dry state. Furthermore, the wash-medium did not support acetylene reduction by the free-living UMKL 76, even with numbers as high as 4×10^{10} rhizobia per ml.

Thus the acetylene reduction observed in these experiments occurred within the cytoplasm of intact protoplasts. Maximum rates of reduction approximated 2-5% of that observed in fresh nodules (and this does not take into account the yield of protoplasts). In other experiments, rates of reduction of 12-13 nmol C₂H₄ produced per mg dry weight per hour were observed, which compares favourably with the reported values of about 46 nmol C₂H₄ produced per mg dry weight rhizobia per h (ref. 21).

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Inhibition by acetylene of conventional hydrogenase in nitrogen-fixing bacteria

ONE of the earliest observations associated with nitrogen fixation was that all nitrogen-fixing organisms contained hydrogenase and nitrogenase¹. We show here that acetylene, which is widely used as a substrate for nitrogenase, inhibits conventional hydrogenase activity and thus prevents the uptake of H₂ formed by nitrogenase in the presence of CO. Our evidence also suggests that azotobacter nitrogenase forms H₂ *in vivo* and that hydrogenase reoxidises this H₂ to provide reducing power for metabolism.

Hydrogenase can be classified into two major types: first, the ATP-dependent hydrogen-evolving capacity of nitrogenase, which accounts, in part, for the above correlation, and second, the hydrogenase which is not dependent on ATP. This second type can be subdivided into the reversible hydrogenases, common in anaerobic bacteria and presumably used as an electron sink to maintain a constant level of oxidised ferredoxin (or equivalent electron carrier), and the unidirectional, hydrogen-oxidising type found in azotobacter^{2,4} and some nitrogen-fixing bacteroids³. Dixon⁵ suggested three possible functions for this unidirectional hydrogenase: (1) as an oxygen scavenger to supplement respiratory protection of nitrogenase; (2) to prevent the H₂ evolved by nitrogenase from inhibiting the enzyme, and (3) enhancing the efficiency of nitrogenase by recycling the evolved H₂. There was little supportive evidence for these possibilities and the failure to observe H₂ evolution by nitrogen-fixing azotobacter under CO and argon, conditions favourable for any such evolution, led Postgate⁶ to suggest that the nitrogenase site was anhydrous *in vivo* and that the hydrogen ion was unable to reach it. Brotonegoro⁷, however, observed that nitrogen-fixing *A. chroococcum* evolved H₂ in the presence of CO if C₂H₂ was also present. We have investigated this observation.

The organisms used in this study were *Azotobacter chroococcum* (N.C.I.B. 8003) and *Klebsiella pneumoniae* M5A1. The growth, harvesting and preparation of crude supernatant extracts from these organisms have been described elsewhere^{7,8}. Whole cells of *A. chroococcum* were obtained from batch cultures (100-ml volumes shaken in 250-ml conical flasks under air at 30 °C) and the *K. pneumoniae* cells were from a glucose-limited chemostat culture repressed for nitrogen fixation⁹.

For the enzyme assays, the nitrogenase activity in crude supernatants⁷ or whole cells¹⁰ of *A. chroococcum* was measured by acetylene reduction or H₂ evolution⁸. Hydrogenase activity in both *A. chroococcum* and *K. pneumoniae* was determined manometrically^{2,11}.

Table 1 shows clearly that acetylene inhibited the ATP-independent H₂-evolving capacity of the reversible hydrogenase of *K. pneumoniae* and the H₂-oxidising capacity of the unidirectional hydrogenase of *A. chroococcum*, but that it did not inhibit the ATP-dependent H₂-evolving capacity of nitrogenase. C₂H₂ seemed to be a more effective inhibitor of hydrogenase than CO, particularly with whole cells of *A. chroococcum*.

Table 2 shows the effect of CO and C₂H₂ on hydrogen evolution by whole cells of *A. chroococcum*. The effect of the inhibitors varied with different batches of cells but 5% CO consistently inhibited acetylene reduction by > 95% and the rate of H₂ evolution was always greater in the presence of both CO and C₂H₂ than with either gas alone. NH₄-grown cells, repressed for nitrogenase activity, did not evolve H₂; this strongly suggests that the enzyme responsible in N₂-grown cells is nitrogenase.

We have confirmed the observation made by Brotonegoro⁷ and extended it to show inhibition of conventional hydrogenase by acetylene. The evolution of H₂ which occurs

Table 1 Inhibition by acetylene and carbon monoxide of hydrogenase activity

Concentration of inhibitor (% C ₂ H ₂ in gas phase)	ATP-independent hydrogenase		Inhibition %		ATP-dependent hydrogenase Crude preparation of nitrogenase from <i>A. chroococcum</i> *
	Reversible hydrogenase from <i>K. pneumoniae</i> Whole cells	Crude supernatant	Unidirectional (H ₂ uptake) hydrogenase from <i>A. chroococcum</i> Whole cells	Crude supernatant	
3.7	67				
6.5	82				
5.0		45	51(7)†	51(36)	0(0)
10.0		85	75	83	0
20.0			96	95	0

K. pneumoniae hydrogenase was measured in NH₄-grown cells and supernatants which contained no nitrogenase activity: H₂-evolving activity of whole cells (2.3 ml, 0.6 mg of protein) with added glucose (46 mM) was measured manometrically at 30 °C under argon. Crude supernatants (2.4 mg of protein) were also assayed manometrically under argon at 30 °C in 70-mM HEPES buffer, pH 7.2, Na₂S₂O₄ (20 mM) and methyl viologen (2 mM) in a final volume of 2.3 ml. The reactions were started by tipping either glucose or Na₂S₂O₄ from the side arm. *A. chroococcum* hydrogenase was measured manometrically at 30 °C by following H₂ uptake in 100-mM phosphate buffer pH 8.0, containing washed cells (0.5 mg (dry weight)) or crude supernatants (3.4 mg protein) with potassium ferricyanide (24 mM) as the electron acceptor. The atmosphere contained H₂ (80%), C₂H₂ as indicated with argon as the normalising gas.

Acetylene also failed to inhibit the H₂-evolving capacity of nitrogenase from *K. pneumoniae* in the presence of CO (Dr B. E. Smith, personal communication).

*The ATP-dependent H₂-evolving capacity of nitrogenase in crude supernatants of *A. chroococcum* was measured in the presence of 20% CO to prevent competition for electrons between the acetylene-reducing site and the H₂-evolving site. Otherwise, the assay conditions were as described by Yates and Planqué⁷.

†The figures in parentheses show % inhibition by 5% CO.

when *A. chroococcum* is treated with both CO and C₂H₂ can now be interpreted simply; CO blocks all but the hydrogen-evolving function of nitrogenase¹² and CO plus C₂H₂, but mainly C₂H₂, blocks the conventional unidirectional hydrogenase which would otherwise reoxidise the hydrogen formed by nitrogenase. In experiments not reported here, the aerobe *Mycobacterium flavum* 301 shows a similar pattern of responses to CO and C₂H₂.

Table 2 H₂ evolution and acetylene reduction by N₂-fixing *A. chroococcum*

Amount of gas added to the atmosphere	C ₂ H ₂ reduced (nmol h ⁻¹)	H ₂ evolved (nmol h ⁻¹)
none	0	0
10% C ₂ H ₂	780	20
5% CO	0	440
10% C ₂ H ₂ +5% CO	5	760

The nitrogenase activity of *A. chroococcum* was measured in 10 ml suspensions of whole cells (0.25 mg dry weight ml⁻¹) in 25-ml conical flasks at 30 °C under a PO₂ of 0.05 atm.

These results given a clear indication that H₂ is formed by azotobacter nitrogenase *in vivo* but, instead of being evolved, it is normally reoxidised by hydrogenase to provide additional reducing power for metabolism. This recycling of H₂ formed by ATP-dependent nitrogenase activity coupled with the ability of H₂ to support oxidative phosphorylation in azotobacter² may partly explain the apparently greater efficiency of nitrogenase in azotobacter compared with *C. pasteurianum*¹³; ATP-independent hydrogenase evolves H₂ during growth of *C. pasteurianum*. Thus, the evidence suggests that possessing hydrogenase improves the economy of N₂-fixing aerobes, so the correlation between hydrogenase and nitrogenase¹ now has a rational basis.

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Linear relationship between phytochrome photoequilibrium and growth in plants under simulated natural radiation

DEVELOPMENT in higher plants is highly responsive to environmental factors, the most important, in natural conditions, being light. Perception of light quality involves at least two photoreceptors, of which phytochrome is the better understood¹. Most work on its function, however, has been carried out with etiolated (that is dark grown) seedlings irradiated from relatively narrow wave band sources. There is no direct evidence on the function of phytochrome in the natural environment². Using simulated natural radiation conditions, we have now found evidence to support the hypothesis that phytochrome acts to detect mutual shading through the changed quality of natural radiation, and to redirect development appropriately³.

Phytochrome is a photochromic protein existing in two interconvertible forms; P_r, which absorbs maximally at 660 nm; and P_{fr}, absorbing maximally at 730 nm (ref. 1). Thus, in continuous irradiation with broad-band sources absorbed by both P_r and P_{fr}, a photodynamic equilibrium is achieved characterised by the steady-state proportions of P_r and P_{fr}. This photoequilibrium is usually determined as P_{fr}/P_{total} and is known as ϕ . We have recently shown that ϕ can be quantitatively related to the ratios of the quantum flux densities at 660 nm and 730 nm (known as ξ) using a range of natural and artificial radiation sources³. In addition, certain parameters of development were shown to be related, through ϕ , to ξ , in a predictable, although non-quantitative, manner³. We report here a direct linear relationship between a growth parameter (the logarithmic stem extension rate) and apparent phytochrome photo-

equilibrium (ϕ) as estimated from quantum flux density ratios (ξ).

In this work seeds of fat hen (*Chenopodium album* L.), a common weed of arable land, were collected from the field, germinated and grown to the third leaf stage in a controlled environment room (16 h, 20 °C day: 8 h, 15 °C night) under radiation from a bank of 80-W Warm White fluorescent tubes providing a quantum flux density of $180 \mu\text{E m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR) as defined by McCree⁴. Seedlings selected for uniformity were then transferred to the light treatment chambers using the photo- and thermoperiod conditions described above.

The experimental light sources were designed to provide equivalent amounts of PAR using 6 × 2 foot 40-W Colour Matching 55 fluorescent tubes. To vary the phytochrome photoequilibrium while maintaining constant PAR, additional far red (> 660 nm) radiation was provided from 60-W unfiltered tungsten bulbs, or from a bank of water-cooled 150-W clear tungsten filament lamps filtered through a red and a green sheet of Perspex. The PAR in all treatments was adjusted to $100 \mu\text{E m}^{-2} \text{s}^{-1}$. The added far red radiation allowed a range of estimated phytochrome photoequilibria between $\phi = 0.71$ and $\phi = 0.29$. The spectral energy distribution of each radiation source was measured and the apparent phytochrome photoequilibria estimated from the ξ values as described previously³.

Figure 1 shows the appearance of two originally identical seedlings after treatment with radiation of equal photosynthetic effectiveness but different ξ values for 21 d. The lower phytochrome photoequilibrium established in the plants given added far red radiation has led to extreme modification of develop-

Fig. 1 Photograph of *Chenopodium album* seedlings grown for 21 d under radiation sources providing equal quantum flux densities in the photosynthetically active regions ($100 \mu\text{E m}^{-2} \text{s}^{-1}$) but with different amounts of far red radiation establishing different phytochrome photoequilibria. The estimated photoequilibria under the two sources are a, 0.71; b, 0.38.

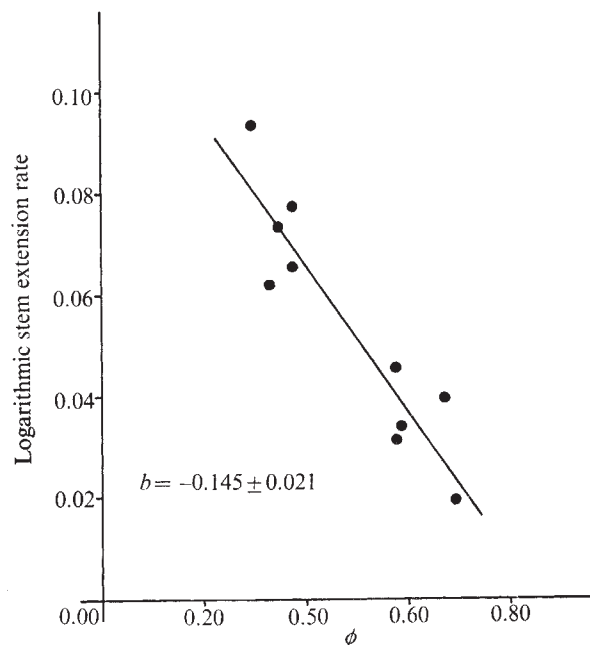
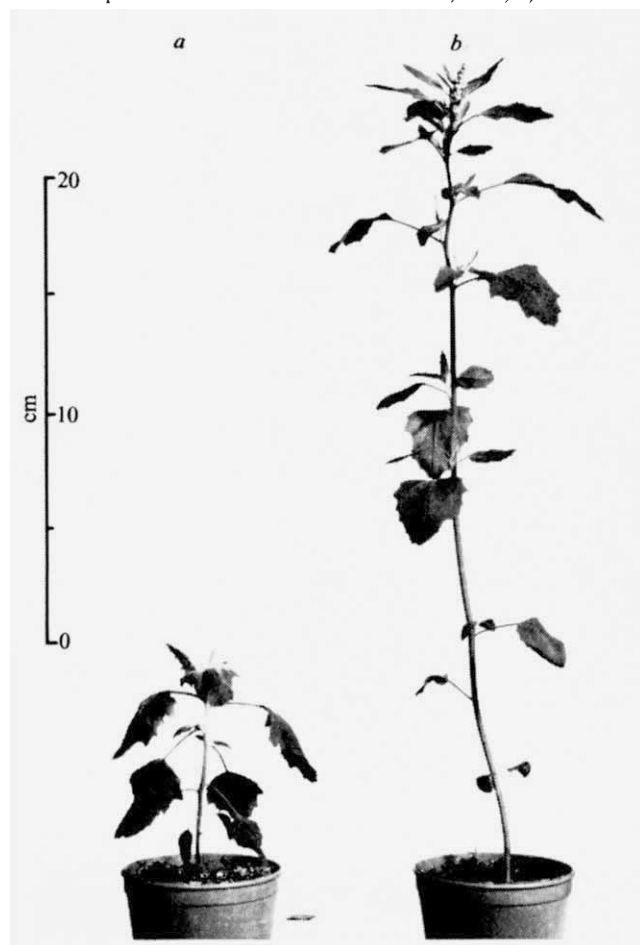


Fig. 2 The relationship between phytochrome photoequilibrium (ϕ) and the logarithmic rate constant of stem extension for *Chenopodium album* seedlings growing under equal amounts of PAR. The line has been fitted by regression analysis, and the correlation coefficient is -0.924 .

mental pattern. In particular, stem elongation, branching, flowering, and leaf pigmentation are modified.

During the early period of growth, *Chenopodium album* seedlings grow exponentially with respect to overall height. When plants were grown under radiation sources providing equivalent PAR but different phytochrome photoequilibria, the logarithmic rate constants of stem elongation were found to be directly, and linearly, related to the estimated phytochrome photoequilibria (Fig. 2). Other parameters of development are also related to ϕ , but the linear relationship with the logarithmic stem extension rate is the most clearcut response noted. Many workers have previously shown that a modification of ϕ at the end of the daily light period has substantial effects on growth⁵⁻¹⁰. In more detailed experiments to be published elsewhere, we have demonstrated that the phytochrome photoequilibrium established during the light period is responsible for at least 80% of the change observed in stem extension rate, and end-of-day effects, although important, are quantitatively much smaller, although again linearly related to ϕ , as first shown by Vince-Prue⁸. We have also shown that the linear relationship presented in Fig. 2 is not merely a quirk of *Chenopodium album*; it has been seen in other species including the stinging nettle (*Urtica dioica*) and rose-bay willow-herb (*Chamaenerion angustifolium*). A survey of sun- and shade-plants is being undertaken to search for systematic variation in responsiveness to light quality.

These data strongly support the hypothesis we previously proposed that the principal function of phytochrome is to detect the quality of light in the red and far red wavelength regions, and thus to perceive mutual shading^{2,3}. For plants adapted to an herbaceous (that is non-phanerophytic) habit, competition for light is extremely important, and a sensitive mechanism for response to mutual shading is clearly of great adaptive value.

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Hydromechanical adaptation in the solitary free-living coral *Fungia scutaria*

THE scleractinian coral *Fungia scutaria* produces a dense, diskoid skeleton that is adapted for stability and abrasion resistance in turbulent water. Water motion rights skeletons at velocities lower than needed to disturb upright corals. Flow of water over the coral is controlled largely by passive hydromechanical adaptations. Previous reports^{1–3} suggest that muscular activity is the main mechanism enabling free-living solitary corals to follow their peculiar way of life. Our work indicates that passive hydromechanical adaptations can be a more important factor in shallow turbulent waters. Several species of free-living corals are known to migrate, avoid burial, and right themselves by action of their polyp^{1,2} or by symbiotic activity of sipunculid worms³. Most other Indo-Pacific solitary corals such as *F. scutaria* are inactive, yet remain upright in turbulent waters. Dead skeletons of *F. scutaria* are generally found with the oral surface upward, and seem to resist erosion far better than other corals. We overturned dead skeletons in one area; they were righted during the next period of heavy waves, suggesting unique hydraulic and structural properties of the skeleton.

Fungia stability was tested on an intertidal bench subjected to wave-induced unidirectional flow of 30–50 cm s⁻¹. Occasional swells swept across the bench and accelerated flow. Between surges *Fungia* skeletons were set out, either upright or inverted. In the wake of the surge, the resting position of each skeleton was noted. Inverted skeletons were righted by single surges of 1–2 m s⁻¹. Overturned skeletons exposed to a single surge were righted 40 out of 50 times, whereas upright corals remained undisturbed in 49 out of 50 trials. The skeletons generally came to rest with their long axis parallel to the direction of current flow, reducing the cross-sectional area presented to the current and increasing stability.

About 70% of *Fungia* found on a reef flat in Kaneohe Bay, Hawaii, rested upright with their long axis aligned within 30° of the local set of storm waves. The hydraulic response of *Fungia* is similar to that of concavo-convex structures such as pelecypod valves in shallow marine environments^{4,5}; in the case of *Fungia*, this response has adaptive significance.

Water flow over the coral surface was observed in a flume. Dye injected from a syringe was used to trace flow. In moving water, dye in contact with living or dead corals moves slowly towards the stomodaeum. The rough surface creates frictional drag and decreases water velocity at the boundary. For example, in a current of 30 cm s⁻¹ (Fig. 1d) water moved at 0.3 cm s⁻¹ on the downstream portion of the coral directly against the current and at 1 cm s⁻¹ on the upstream portion of the disk. The radial exert septae have a major role in directing and reducing flow. Dead skeletons and living corals with contracted polyps direct flow into the stomodaeum (Fig. 1d); corals with expanded polyps or dead skeletons smoothed with cement (Fig. 1e) do not so direct the flow. In cross section *Fungia* resembles an airfoil, and a moving fluid induces a low pressure area over the stomodaeum. Reduction in current velocity at the coral surface permits flow into this area of low pressure and probably facilitates ciliary control of the surface film⁶ in all current conditions.

The ability of the living polyp to right the skeleton on various

substrata and the role of burrowing crabs in the process were tested in continuous flow aquaria. Live corals and dead skeletons in a graded series of 10–100 g wet weight were placed upside down on the substrata (Table 1). Substrata used were sand, gravel and hard bottom. One experimental series included approximately 15 xanthid crabs (carapace width 3 cm). The other series excluded crabs. Although many of the live *Fungia* eventually righted themselves on the rubble substratum, the process took several days (compared with minutes for more active species^{1,2}). Corals on sand and hard substratum did not right themselves. Crabs had a minor role in righting the corals, overturning two dead skeletons.

Ability to migrate to the surface was tested by burying ten living *Fungia* under 1 cm of sand. No coral reached the surface; all were dead at the end of 7 d. Strong currents and wave motion in the typical shallow water *F. scutaria* habitat causes erosion; the coral must avoid abrasion by moving rubble, overturning, and physical removal rather than *in situ* burial. Unlike its relatives which inhabit calm deep water (where sediments eventually come to rest), this species does not ordinarily encounter thick deposits of fine sediment.

Resistance to abrasion was tested for the four most abundant coral species in the area (Table 2). Live corals were tagged and the skeletal weight measured using the buoyant weighing technique⁷. The corals were placed in a cement mixer containing screened carbonate gravel of 2 cm diameter and flowing seawater. The drum of the mixer was then rotated 20 times in 1 min. The corals and abraded fragments were returned to a continuous flow aquarium and reweighed. The test fragmented the branched

Fig. 1 *F. scutaria* in unstable overturned position will begin rolling when subjected to strong current surge (a), presenting a larger cross section to water flow. This rights the coral (b, c) and leaves it in a stable position (d). Flow patterns over the surface of a living coral with a contracted polyp (d) are compared with that of the coral with an expanded polyp (e).

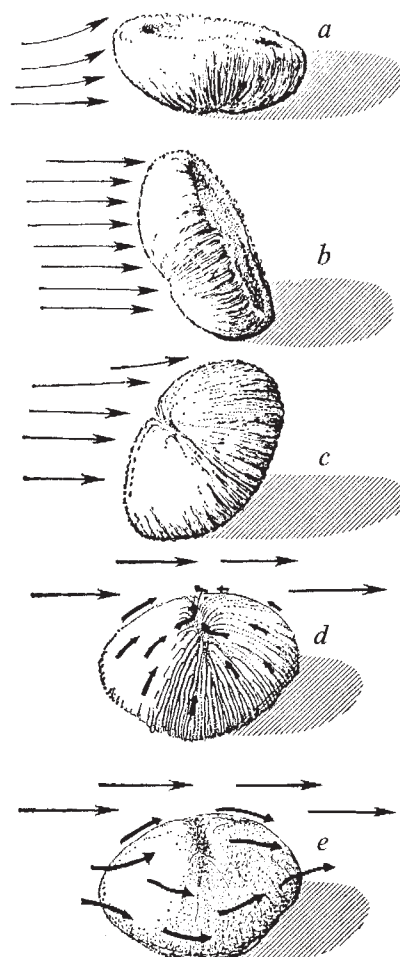


Table 1 Ability of *F. scutaria* to right themselves

	Position after 7 d			
	Live corals		Dead coral skeletons	
	Inverted	Upright	Inverted	Upright
Hard bottom with crabs	9	1	10	0
Hard bottom with crabs	9	1	10	0
Hard bottom without crabs	9	1	10	0
Hard bottom without crabs	10	0	10	0
Rubble with crabs	4	6	9	1
Rubble without crabs	3	7	10	0
Sand with crabs	10	0	9	1
Sand without crabs	10	0	10	0

colonial species and removed all tissue on exposed surfaces. *Fungia* remained intact and lost only a small amount of tissue and skeletal material on the septal margins and costal spines. The septocostal ornamentation fends off the abrasive rubble and protects exposed tissue.

Fungia lost only 1% of its skeleton compared with 40–80% loss for the other species (Table 2). Only the bases of the colonial species remained intact. Tissue regeneration in *Fungia* was complete in 5 d. Colonial corals and abraded fragments lost most of their tissues and died within a few days. *Porites* and *Montipora* eventually showed slight regeneration on the damaged bases, but approximately 90% of the original tissue area was killed.

Table 2 Abrasion resistance, density and cross sectional area/weight ratio of common Kaneohe Bay corals (five corals of each species)

Species	% Loss in abrasion test		Dry skeletal density (g cm ⁻³)		Cross-sectional area/buoyant weight ratio (cm ² g ⁻¹)	
	Mean	s.d.	Mean	s.d.	Mean	s.d.
<i>F. scutaria</i>	1.4	0.6	1.86	0.06	0.25	0.03
<i>Montipora verrucosa</i>	38.8	9.5	1.47	0.12	1.36	0.16
<i>Porites compressa</i>	47.4	17.1	1.53	0.06	0.59	0.12
<i>Pocillopora damicornis</i>	79.6	6.8	1.70	0.17	0.64	0.16

Fungia has the highest skeletal density of the species tested. It also shows a very low cross sectional area per unit buoyant weight, producing a stable low drag profile in moving water (Table 2). Specimens weighing as much as 3 kg have a centre of gravity within 2 cm of the substratum. Branched species of similar mass have a centre of gravity at least 5 cm above the substratum, leading to instability and a need for attachment. The high density of *Fungia* and its low surface-to-volume ratio⁸ enhance abrasion resistance.

Thus, in addition to use of muscular activity, hydromechanical adaptation is a significant factor in many species of solitary free-living corals. *F. scutaria* is typical of solitary shallow water fungiids which have evolved hydrodynamically stable abrasion resistant skeletons. These corals passively use the surrounding energy field of moving water to their advantage, eliminating the need for a strong, muscular, active polyp.

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Reshedding of *Toxoplasma* oocysts by chronically infected cats

INFECTION with *Toxoplasma gondii* is prevalent in man and animals. It can be acquired congenitally by the foetus from an infected mother¹, but also throughout life by eating undercooked meat² or by contact with contaminated faeces of cats³. All forms of *Toxoplasma* lose their infectivity quickly when exposed to the environment, with the exception of oocysts shed in feline faeces. These are remarkably resistant to ordinary environmental influences and can survive in most soil for several months⁴. In the acute phase of infection cats may shed several million oocysts in 1–2 weeks. It has been assumed that after the acute phase, chronically infected cats are safe as pets⁵ since they have acquired immunity and oocysts seldom pass in the faeces^{6–8}. I report here the reshedding of *T. gondii* oocysts by chronically infected cats in the absence of exogenous reinfection.

Twelve cats (four litters) were raised by their mothers from birth in my laboratory. Daily faecal examinations from birth did not reveal coccidian oocysts. Cats were weaned and bled for antibody determination at 83–106 d of age. Antibody to *Toxoplasma* was not detected in the sera of weaned cats by the Sabin–Feldman dye test. After weaning, all 12 cats were housed individually in stainless steel cages. Ten cats (nos 3–12) were fed 10⁵ cysts of the M-7741 strain of *T. gondii* on day 0. Cats 1 and 2 were not fed *Toxoplasma* and served as controls. The faeces of all cats were examined daily for coccidian oocysts⁹. All 10 inoculated cats shed millions of *T. gondii* oocysts between 4 and 20 d after infection with *Toxoplasma* (Table 1). One cat (no. 7) reshed oocysts on day 28 after infection (Table 1). Three littermate cats (nos 9, 10, 12) spontaneously shed *Isospora felis* oocysts (Table 1). The remaining cats did not shed *I. felis* oocysts. The source of *I. felis* infection remained unknown.

To investigate interrelationships between *T. gondii* and other feline coccidia the animals were divided on day 30 after infection with *Toxoplasma* into two groups of six (A and B). Cats in group A (nos 1, 4, 6, 8, 10 and 11) were fed 10⁴ sporulated oocysts of *I. rivolta* on day 30 after infection with *Toxoplasma* and 10⁴ *I. felis* 13 d later. The effect of superinfection with *I. felis* and *I. rivolta* on established *Toxoplasma* infection seemed of particular significance in the light of Chessum's¹⁰ report that a cat infected with *T. gondii* for 3 months started reshedding *Toxoplasma* oocysts 10 d after it was fed *I. felis* oocysts. *I. felis* and *I. rivolta* infections are asymptomatic in cats¹¹.

Both *I. rivolta* and *I. felis* caused patent infections in the inoculated cats. *I. rivolta* oocysts were shed between 6 and 12 d after *I. rivolta* was fed to the cats and *I. felis* were shed between 8 and 25 d after *I. felis* was fed to them. The cats in group B did not receive *I. felis* or *I. rivolta* and did not shed these organisms during this period. Four of five *Toxoplasma*-infected cats in group A reshed *T. gondii* oocysts beginning on day 10 after feeding with *I. felis* (or day 53 after infection with *Toxoplasma*); none reshed *I. rivolta*. Only one cat (no. 9) from group B reshed *T. gondii* oocysts during this period (Table 1).

On day 61 after infection with *Toxoplasma* cats in group B (nos 2, 3, 5, 7, 9 and 12) were fed 10⁵ *I. felis* oocysts. All five *Toxoplasma*-infected cats (nos 3, 5, 7, 9 and 12) reshed *T. gondii* oocysts starting 9–14 d after feeding *I. felis* (Table 1). Cats 9 and 12 that were spontaneously infected with *I. felis* shed fewer *Toxoplasma* oocysts than did cats 3, 5 and 7 that were not previously infected with *I. felis*.

Overall, 9 of 10 cats reshed *Toxoplasma* oocysts. The reshed oocysts were identified as *T. gondii* by mouse to mouse passage.

Table 1 Shedding of *Toxoplasma* oocysts by cats with or without *I. rivolta* and *I. felis* infection

Cat no.	Group	Primary Age of cats (d)	<i>Toxoplasma</i> infection Days oocysts shed	No. of oocysts shed	<i>I. rivolta</i> fed PTID 30	<i>I. felis</i> fed PTID 43	Reshedding of <i>Toxoplasma</i> oocysts		<i>I. felis</i> fed PTID 61	Reshedding of <i>Toxoplasma</i> oocysts	
							oocysts shed	No. of oocysts shed		oocysts shed	oocysts shed
1*	A	89	None		Yes	Yes	None		No	None	
2*		89	None		No	No	None		Yes	None	
3	B	99	5-18	10 ⁸⁻¹⁹	No	No	None		Yes	72-84	10 ⁷⁻²²
4	A	99	4-17	10 ⁷⁻³⁸	Yes	Yes	53-68	10 ⁶⁻⁵⁷	No	None	
5	B	99	5-18	10 ⁷⁻²⁷	No	No	None		Yes	70-85	10 ⁷⁻¹⁷
6	A	106	4-15	10 ⁷⁻⁵⁰	Yes	Yes	53-63	10 ⁶⁻²⁴	No	None	
7	B	106	5-16	10 ⁷⁻⁸⁶	No	No	28	10 ^{3†}	Yes	70-75	10 ⁶⁻⁶⁹
8	A	106	5-20	10 ⁸⁻¹³	Yes	Yes	53-65	10 ⁶⁻³⁴	No	None	
9†	B	83	4-11	10 ⁷⁻¹⁴	No	No	31-33, 50	10 ^{4‡}	Yes	75-76	10 ^{4‡}
10†	A	83	5-16	10 ⁸⁻¹³	Yes	Yes	None		No	None	
11	A	83	4-14	10 ⁷⁻⁴³	Yes	Yes	53-64	10 ⁶⁻¹⁷	No	None	
12†	B	83	4-15	10 ⁷⁻¹⁵	No	No	None		Yes	72-77	10 ⁵⁻²¹

PTID, post-*Toxoplasma* infection day. All cats except nos. 1 and 2 were fed 10⁶ cysts of the M-7741 strain of *T. gondii* on day 0. Faeces of cats in group A were examined for 1-74 PTID and of group B for 1-85 PTID. Littermates are bracketed together.

*Cats 1 and 2 remained free of *Toxoplasma* infection throughout the experiment.

†Spontaneously infected with *I. felis* (cat 9 shed *I. felis* on days 2, 3; cat 10 shed 25-34, cat 12 shed 25-34, 9-20 PTID).

‡Estimated number of oocysts in daily sample. Only one or few oocysts seen in faecal-float preparation.

Reshedding of *T. gondii* oocysts by 9 of 10 cats within a short time (9-14 d) after feeding *I. felis* suggests a reactivation of latent *Toxoplasma* infection, probably by interfering with immunity to toxoplasmosis in the feline intestine.

Between 20 and 64% of cats in several surveys contained antibody to *T. gondii*^{1,2}, indicative of exposure to the organism. Many of these cats are presumably permanent carriers of *Toxoplasma*. As *I. felis* is also a common parasite of cats the present findings may have a public health significance, especially in homes with pregnant women. It is sound advice for pregnant women to handle cat faeces with care irrespective of the *Toxoplasma* infection status of the cat³.

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has not been verified. Obviously, the production of a highly susceptible strain of triatomine bug that would provide a quick and accurate diagnosis of chronic Chagas' disease would be a distinct advantage. I report here on the selection of both susceptible and refractory lines of *Rhodnius prolixus*, and the results of this selection programme are related to the genetic control of susceptibility to infection with *T. cruzi*.

Two strains of *T. cruzi* were chosen for their differing characteristics. The Peru strain was originally isolated in 1963 and after passage in mice was stabilized by cryopreservation⁶. This highly virulent strain has a prepatent period of 8-14 d in mice, and is lethal at 25-30 d following inoculation of more than 100 organisms⁷. In contrast, strain 7, isolated in Brazil in 1965 and stabilized after passage in mice, is of low virulence. This strain also has a pre-patent period of 8 d, but, unlike the Peru strain, parasitaemia declines about 30 d after inoculation, and the mice generally recover.

To study the effect of selection on susceptibility in *R. prolixus* over successive generations, a standard system was used to infect the bugs and, later, to measure the infection. TO mice were first used to provide a non-infecting feed, given 28 d before the infecting blood meal. Six- to eight-week-old male mice of the same strain were then infected with one or other strain of *T. cruzi* and used to feed the bugs which had moulted to fifth instar larvae. Feeding took place either 14 d after infection of the mice with the virulent Peru strain or 21 d after infection with the less virulent *T. cruzi* strain 7. In both cases the mice were checked for patent parasitaemia before feeding commenced. Bugs were kept individually in glass tubes and maintained in incubators at 28 °C and 70% relative humidity. Thirty five days after infection, adult bugs were again fed on clean mice, and faeces produced by the bugs within 1 h of feeding were collected in 0.2 ml Hanks solution. Bugs which did not feed were removed from the experiment. The number of trypanosomes excreted in a 20 µl sample of the mixed faecal-Hanks suspension was counted by systematically scanning 100 fields (×450). In the absence of detectable parasites, the bug was given a further clean feed 14 d later, and the second faecal sample was examined. If this was again negative, the remaining faecal suspension was injected intraperitoneally into a non-infected mouse, and blood samples examined 14 and 21 d later.

Where trypanosomes were seen at the first examination of faeces, the score represented the number counted in 100 fields. If a bug showed no evidence of infection on all tests it was scored as zero, whereas if positive at either the second (faecal) or third (blood) test it was assigned an

Inheritance of susceptibility to *Trypanosoma cruzi* infection in *Rhodnius prolixus*

MILLIONS of cases of Chagas' disease exist in Central and South America¹. Apart from being virtually incurable, a major problem in this disease is symptomless carriers harbour trypanosomes all their lives but it is often difficult to demonstrate the parasite². At present, the simplest and most sensitive method of diagnosis³ is to feed uninfected triatomine bugs on the suspected case and subsequently to examine a faecal sample from the bug for the presence of trypanosomes. There is, however, great variation both within and between species of Triatominae, in their susceptibility to infection with *Trypanosoma cruzi* (see, for example, refs 4 and 5), and though a genetic basis for this variation in susceptibility has been suggested⁴, it

arbitrary score of 1. In all, 1,725 bugs were reared, given an infecting feed, and scored in this way.

Selection was subsequently made from the two parental populations of bugs on the basis of individual faecal trypanosome scores. A single-pair full-sib mating system was followed for both groups of bugs, resulting in 19 F_2 families in the Peru strain group, while in the strain 7 group selection was carried one generation further to produce 17 F_3 families.

Results showed that selection did not significantly increase the proportion of refractory (score zero) bugs in lines selected for refractoriness. This suggests that refractoriness to infection with *T. cruzi* is not controlled by alleles at a single locus in *R. prolixus*. By contrast, a quantitative analysis of faecal scores (transformed to a log ($n+1$) scale), revealed that the intensity of infection sustained by individual bugs is a heritable characteristic. First, significant difference ($P<0.001$) were found between bugs of the same generation infected with the two different strains of *T. cruzi*. Bugs fed on strain 7 infected mice were found to have a mean faecal score almost twice as high as that of bugs infected with the more virulent Peru strain. Clearly, strain differences affect the intensity of infection of individual bugs as well as affecting susceptibility rates^{4,8} (that is, proportions of infected bugs in a population).

Further analysis of F_1 and F_2 families of *R. prolixus* infected with different strains of trypanosome showed significant differences in faecal scores between sexes ($P<0.001$), when tested against the considerable variation between families. The same was true of F_3 families infected with strain 7. Control weighing experiments to determine any possible effect of variation in the size of blood meal or faecal sample were negative. There was no significant dif-

ference in blood-meal size between male and female fifth instar larvae, although it was interesting to note that adult females took significantly larger blood meals than males and also excreted a significantly greater proportion of their meals within 1 h of feeding. Thus, it might be anticipated that adult females would have higher scores than males, but that is not the case. In fact, male scores were significantly greater than female scores in all generations tested. Miles *et al.* failed to detect such a sex-difference, but their conclusion was based on tests with a small number of bugs. Significant differences in faecal scores were also found between families of all generations infected with either strain of trypanosome, reflecting a resemblance between the intensity of infection of related bugs—one of the basic characteristics of metric traits⁹.

Figure 1 shows that selection for high and low levels of intensity of infection was successful. Analysis of F_3 family scores showed that differences between the selection lines were highly significant for both sexes ($P<0.001$) when tested against the variation between families within lines. The realised heritability of intensity of infection in *R. prolixus* was calculated from the regression of response (r) on selection differential (s)¹⁰. The regressions of both selection lines being highly significant ($P<0.001$), the divergence of the two slopes provided an estimate of 5.5% for the heritability of this character. This low value relates to this laboratory colony and could differ for a natural population⁹.

Knowledge of genetic mechanisms controlling the susceptibility of insect vectors to infection has so far been limited to mosquitoes and their malarial and filarial parasites, where to date only major gene control mechanisms have been demonstrated^{11,12}. There have been attempts to quantify mosquito susceptibility to *Plasmodium gallinaceum* by counting oocysts¹³, but significant parent-offspring regressions could not be shown. The present work has clearly shown that intensity of infection with *T. cruzi* in *R. prolixus* is a metric character of low heritability, which is sex linked or at least sex limited. For the purposes of aiding the diagnosis of chronic Chagas' disease, it would be desirable to breed a colony of bugs that are highly susceptible to the multiplication of the few trypanosomes ingested from such patients. The present results indicate that using only male bugs of a susceptible stock for xenodiagnosis should enhance the sensitivity of this diagnostic test for chronic Chagas' disease.

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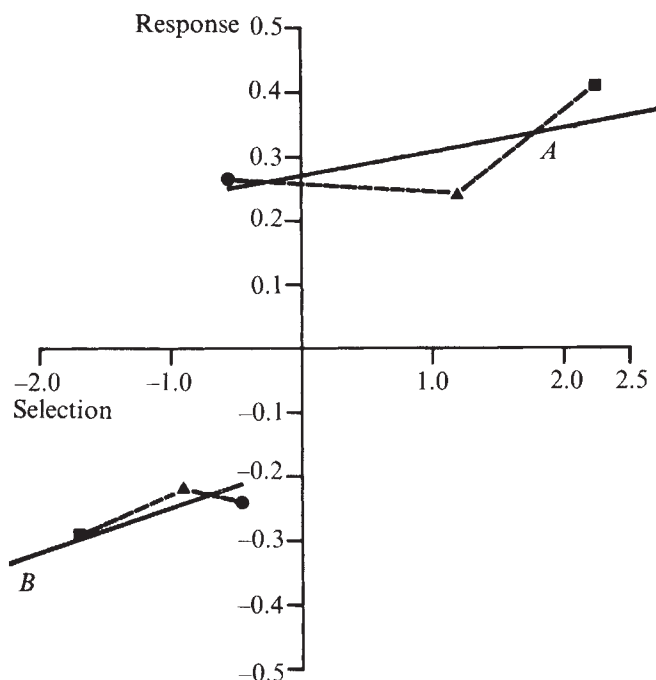
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Fig. 1 Response to two-way selection for intensity of infection with *T. cruzi* strain 7 in *R. prolixus*. Cumulated selection differentials for each generation (weighted according to the number of progeny scored) are plotted against the response to selection derived from faecal scores transformed to log ($n+1$) scale, sexes averaged. Regressions, calculated from 4 high and 5 low selection lines, show the realised heritability to be $3.6\pm1.1\%$ for increases in intensity of infection and, for reductions in intensity of infection, $7.3\pm0.9\%$. This asymmetric response is a common feature of two-way selection programmes¹⁴. ●, F_1 family means; ▲, F_2 family means; ■, F_3 family means. A, $b = 0.0368\pm0.0115$; B, $b = -0.0731\pm0.0091$.



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Functional inactivation of suppressor T cells by heat-killed macrophages

We have shown that peritoneal exudate macrophages, killed by heating at 56 °C for 30 min, can markedly suppress the antibody response to heterologous erythrocytes in primary Mishell-Dutton cultures¹. This heat treatment does not destroy the ability of the macrophages to form rosettes with sheep red blood cells (SRBCs) in the presence of cytophilic antibody, suggesting that some membrane surfaces remain undamaged. The heat treatment, however, does prevent the macrophages from excluding Trypan blue dye, indicating that the cells are no longer viable. Treatment of the macrophages at higher temperatures or with 0.1% glutaraldehyde destroys both their ability to form rosettes and to suppress Mishell-Dutton cultures. Macrophage membranes, semi-purified by separation on sucrose gradients, can act similarly—that is, suppress primary Mishell-Dutton cultures and form rosettes with SRBCs in the presence of cytophilic antibody (W.P. and R.K.G., unpublished). The heat-killed macrophages do not diminish the viability of the cell cultures even when they are maximally suppressive. We have interpreted these results to indicate that the dead macrophages suppress the immune response in culture by absorbing out, and thus rendering inoperative, some factors which are necessary for the cell interactions which result in the generation of plaque-forming cells. This contention is supported by the ability of the heat-killed macrophages to absorb out the helper activity found in the supernatants of mixed lymphocyte reactions (W.P. and R.K.G., unpublished). We reasoned that, if macrophages could absorb interaction factors, they might be able to absorb suppressive factors if they were added to a culture in which suppression was occurring and thus augment the response of suppressed but not normal cultures.

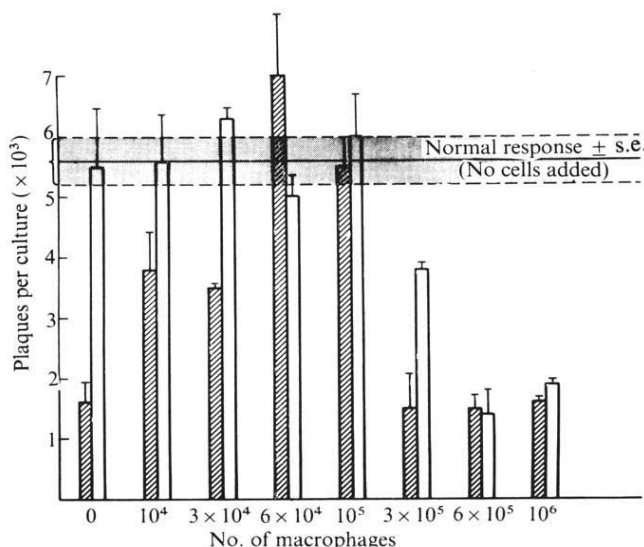
We used a newly described *in vitro* technique to generate educated spleen cells with the capacity to specifically suppress the *in vitro* response to SRBCs (ref. 2). The suppression is mediated by T cells and is antigen-specific when less than 10^6 educated cells are added to spleen cell cultures. We cultured 10^7 BDF₁ spleen cells with 2×10^6 SRBCs according to the Mishell-Dutton technique. Four days later we retrieved the educated cells from culture and added different doses of them to new primary Mishell-Dutton cultures along with 2×10^6 SRBCs. Varying numbers of heat-killed macrophages were included in the second cultures which were incubated for a further 4 d before assay for plaque-forming cells. The results of one such experiment are presented in Fig. 1. The horizontal line represents the response of normal cultures without educated cells or macrophages. Addition of 10^5 educated cells to 10^7 normal spleen cells markedly suppressed the anti-SRBC response, whereas addition of 10^4 educated cells had no effect and served as a control for the effect of adding educated cells. The addition of 10^4 or 3×10^4 heat-killed macrophages to the suppressed cultures produced a moderate augmentation of the response, whereas it had no effect on the non-suppressed culture. The addition of 6×10^4 or 10^5 macrophages to the suppressed cultures brought their response back to approximately normal levels. Again this number of heat-killed macrophages had no effect on the non-suppressed response. Adding 3×10^5 , 6×10^5 or 10^6 heat-killed macrophages suppressed the response in all cultures. Interestingly, increasing the macrophage dose beyond 3×10^5 or 6×10^5 cells did not increase the amount of suppression. This plateau effect is commonly seen (ref. 1 and W.P. and R.K.G., unpublished) and indicates that there is a base level of the immune response which the heat-killed macrophages cannot suppress; on the other hand, live macrophages can suppress the response to a 0 level.

Thus, there seems to be a differential susceptibility of suppression and help to the effect of heat-killed macrophages. Low numbers of macrophages can preferentially abrogate suppression but cannot bring the response significantly higher than the normal response level. When the number of macrophages is high enough to suppress the control cultures, they also suppress the cultures which contain the suppressor cells, probably acting in the latter situation by interfering with help rather than by augmenting suppression. Whether these differential effects are due to different sensitivities of suppressor and helper factors, differences in their affinities for macrophage receptors or different numbers or types of receptors and/or macrophages is at present under investigation.

Since heat-killed peritoneal exudate macrophages do not augment primary Mishell-Dutton cultures at any dose, and can reverse suppression when known suppressor cells are added to the culture, as we have shown here, our results suggest that some mediators of suppression may have affinity for macrophage surfaces. Heat-killed macrophages may prevent their interacting with their target cells by absorbing them from the culture. Of course, the vast body work of Feldmann³ documenting the importance of the macrophage membrane as a site for lymphocyte interaction supports this contention.

Independent of the exact explanation for the phenomenon we have described, the results we have presented have practical as well as theoretical significance. They indicate that one can use this *in vitro* technique to determine whether a poor response is due to a lack of stimulation or to suppression. One can titrate in heat-killed macrophages and if these cells result in an increase of the response, one can be reasonably certain that the response of the test cells is due to alleviation of suppression. The heat-killed macrophages have no inherent augmentory capacity of their own. Note also that the failure of macrophages to augment a response cannot be taken as evidence that suppression is not present. We do not know whether, in other systems, one would find the differential susceptibility of suppression and help that we found in this system. We have found, however, that heat-killed macrophages can augment the generation of cytotoxic killer cells to syngeneic tumour

Fig. 1 Functional inactivation of suppressor cells by heat-killed macrophages. Educated cells were generated by culture of spleen cells *in vitro* with 2×10^6 SRBCs. The normal response to SRBCs of the assay cultures (horizontal line) was suppressed by 10^5 educated cells (hatched columns), whereas 10^4 educated cells (open columns) induced no suppression and served as the control. Increasing numbers of heat-killed macrophages were added to the suppressed or control cultures.



cells *in vitro* (C. Metzler, W.P. and R.K.G., unpublished), suggesting that the generation of suppressor cells is one of the factors contributing to the difficulty in getting a good killer-cell response against tumour cells *in vitro*. In any case, the ability to use heat-killed macrophages in *in vitro* responses to document the presence of suppression in the cultures should be of help in dissecting out some of the complicated interactions between subpopulations of lymphocytes which control the immune response.

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Onset of acetylcholine sensitivity and endplate activity in developing myotome muscles of *Xenopus*

THE distribution of acetylcholine receptors and the onset of functional innervation at the neuromuscular junction during development have been much investigated^{1–3}, as have the events during regeneration and reinnervation of muscle⁴. Although the first appearance of acetylcholine (ACh) sensitivity of the membrane has been charted in chick and mouse myoblasts differentiating in tissue culture⁵, previous studies *in vivo* have either not tested for the initial appearance of ACh sensitivity², or found the muscle membrane to be already sensitive to externally applied ACh¹. In the present study on *Xenopus* embryos we have followed the initial appearance of membrane depolarisation in response to applied ACh and the onset of functional innervation in differentiating myotome muscle *in vivo*.

Myotome formation from the presumptive mesoderm begins before the neural tube closes (stage 17)⁶, and continues up to late swimming stages. Figure 1 shows the arrangement of cells in the presumptive mesoderm and the fully formed myotomes described by Hamilton⁷; a new myotome is formed about every 20 min. The myotomes are innervated segmentally by cells in the ventrolateral part of the neural tube^{7,9}. Innervation, like myotome formation, begins at the

head end and proceeds tailwards. Flexions of the body first occurred between stages 21 (8 somites) and 24 (12–15 somites), the precise time varying between clutches of eggs.

Membrane potentials were recorded with microelectrodes filled with 0.8 M potassium citrate (resistance >100 M Ω ; tip potential <–8 mV). Embryos (stages 19 to 25) were pinned out in a small bath and superfused with Ringer solution (NaCl, 120 mM; KCl, 2.5 mM; CaCl₂, 2 mM; Tris, 2 mM; pH 7.4). The ectoderm was peeled away and the dermatome layer of the mesoderm left intact above the myotomes. The microelectrode was pushed through the dermatome into the muscle cells. Because cells in the myotome have membrane potentials 40 mV greater than those of the dermatome¹⁰ and because the two cell layers are not electrically coupled to each other¹⁰, the position of the microelectrode was never in doubt.

Figure 2 shows records taken in 1 of 37 experiments to test for acetylcholine sensitivity of the muscle membrane. The microelectrode was inserted while an embryo at stage 21 was superfused with Ringer solution. At 10-min intervals the bathing fluid was changed to Ringer containing 10^{–4} g ml^{–1} ACh, which should produce a maximal response; if no depolarisation had resulted after several minutes, Ringer solution was readmitted. Figure 2a illustrates the last test in which ACh was without effect, suggesting that the membrane did not yet contain functional ACh receptors. Figure 2b shows the prompt and rapid depolarisation of 44 mV produced by admission of ACh 9 min later. Subsequent tests gave depolarisations of similar time course and magnitude. The results of this and eight other experiments with test intervals of between 10 and 30 min suggest that ACh receptors become functional relatively quickly. On all occasions the first depolarisation produced by ACh was as great as that produced by subsequent tests, suggesting that the response is immediately established at its maximum level. In 23 other experiments ACh sensitivity was already present when testing began.

The depolarisation in response to bath-applied ACh was always relatively prompt and brought the membrane potential to between –12 and –5 mV, close to the value recorded in adult amphibian muscle, suggesting that the reversal potential, and therefore the underlying permeability changes, are the same as in adult muscle¹¹.

After the appearance of ACh receptors, random deflections of the membrane potential were recorded, indicating that spontaneous liberation of ACh from innervating motor nerves had begun. The interval between these two events was variable, ranging from 15 min to about 1 h; once endplate activity could be recorded, bath application of ACh always produced depolarisation. The initial potentials were small, possibly miniature endplate potentials produced by spontaneous release of ACh without electrical activity in the nerve. A period when extremely large spontaneous depolarisations (up to 60 mV) were recorded invariably followed. Figure 2c shows a pen record of such activity to-

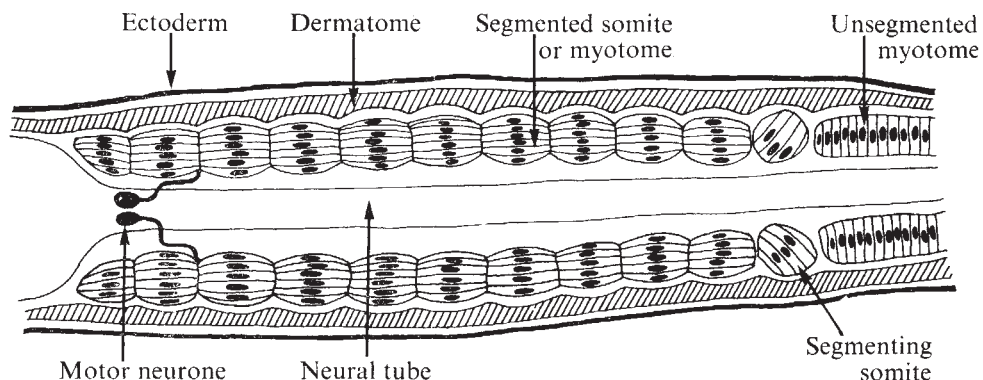


Fig. 1 Diagram of horizontal section through 10 somite *Xenopus* embryo to show arrangement of myotome cells.

gether with the depolarisation produced by superfusion with ACh. A high gain oscilloscope record (Fig. 2d) shows these large depolarisations to take the form of summated endplate potentials. This activity seems most likely to be the consequence of action potentials in the motor nerve since very large miniature endplate potentials are not normally seen in developing neuromuscular junctions¹, and the input resistance of the electrically coupled muscle fibres is not particularly high. Both the spontaneous activity and the response to ACh were blocked by curare. Because electrical coupling between the muscle fibres is good¹⁰, it was not possible to distinguish how many motor nerves contributed to the recorded spontaneous activity. Direct nerve stimulation is not possible in this preparation. Mn^{2+} abolished the endplate potentials, but not the response to perfusion with ACh. Figure 2e illustrates the ACh depolarisation in a preparation made silent by the addition of 2 mM Mn^{2+} to the bathing fluid.

Muscle action potentials were sometimes recorded before any visible endplate activity, although they usually appeared after spontaneous liberation of acetylcholine from the nerve had begun. In spite of high resting membrane potential, the earliest action potentials did not initiate muscle contraction, which was never seen before spontaneous endplate activity could be recorded. These findings make the suggestion that early flexions *Xenopus* embryos are myogenic in origin⁹ extremely unlikely. It seems more probable that early movements are initiated by discharge from the motor nerves and achieved by transmission of the depolarisation from one myotome to the next along the low resistance intercellular pathway, as described by Blackshaw and Warner¹⁰.

These experiments show that formation of the first functional neuromuscular junctions in *Xenopus* takes place between segmentation of the fourth and fifteenth myotome. The sequence of events is summarised in Fig. 3, where the appearance of functional ACh receptors has been put at stage 21½, with flexions beginning at stage 24 (about 2 h later). The onset of this sequence varied from clutch to

Fig. 2 Membrane potential records taken during tests for acetylcholine sensitivity of myotome muscle membrane. *a*, Embryo stage 21: 10^{-4} g ml⁻¹ ACh has no effect on the resting potential of a myotome muscle cell. $E_m = -70$ mV. *b*, Same embryo 9 min later: 10^{-4} g ml⁻¹ ACh now produces large depolarisation. *c*, Embryo stage 24: spontaneous depolarising activity in muscle cell together with response to 10^{-4} g ml⁻¹ ACh. $E_m = -75$ mV. *d*, Oscilloscope record to show spontaneous activity takes the form of summated endplate potentials. Note two miniature e.p.s. on rest of trace. Stage 23: *e*, Depolarisation produced by 10^{-4} g ml⁻¹ ACh in preparation made silent by addition of 2 mM Mn^{2+} to the bathing fluid.

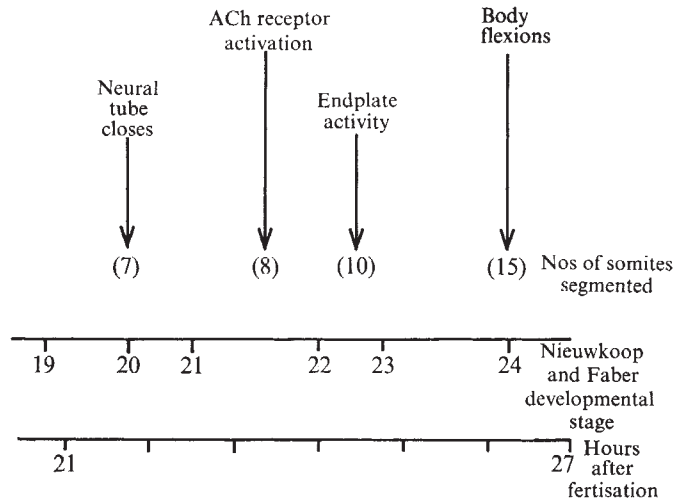
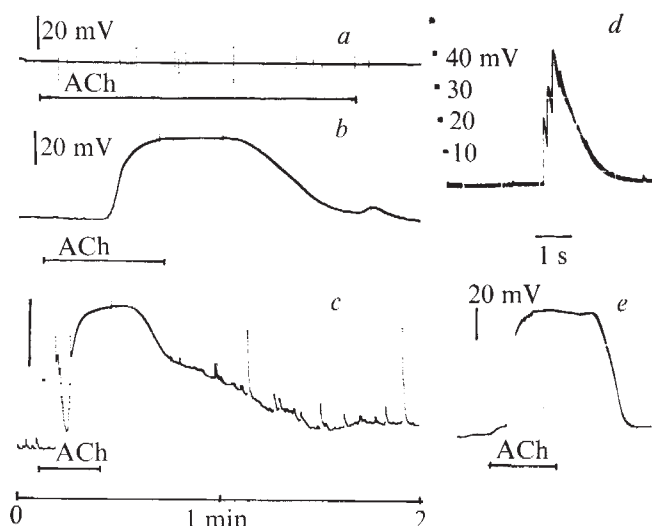


Fig. 3 Diagram to show sequence of events during differentiation of functional neuromuscular junctions in myotome muscle of *Xenopus*. Action potential generation in the muscle membrane may appear anywhere between stages 19 and 23.

clutch so that receptor appearance could occur as early as stage 19 with flexions following 2 h later at stage 21. On occasion however, the whole sequence from the appearance of ACh sensitivity was compressed into as little as 0.5 h. Thus both the timing of the initial insertion of ACh receptors and the length of time taken to establish functionally innervated, contracting muscle showed considerable variation from embryo to embryo. This implies that the development programme does not specify a precisely interrelated timing schedule for this sequence, suggesting that each event is to some extent independent.

The abrupt initial appearance of functional ACh receptors and the speed of subsequent events culminating in the establishment of the first neuromuscular junction, seems, at first sight, surprising. The overall speed of development of *Xenopus*, however, which moves from fertilised egg to flexing tadpole in only 27 h, makes a fast rate of this, and all other developmental sequences, inevitable.

The appearance of spontaneous endplate potentials could mark the time when the nerve ending first makes contact with the muscle. On that view acetylcholine sensitivity in the muscle membrane is established some time before the arrival of the nerve ending and could, therefore, serve in some way to guide the nerve to its final destination. The results are equally compatible with the view that the release of ACh does not begin until some time after the arrival of the nerve, functional connection being a relatively late event in the formation of the neuromuscular junction. Detailed anatomical studies, correlated with electrophysiological studies of the kind described here, might enable this point to be resolved.

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Differential circadian rhythms in pineal and hypothalamic 5-HT induced by artificial photoperiods or melatonin

CHANGES in daylength are principally responsible for synchronising breeding with time of year in mammals and birds¹: for example, ferrets begin to breed in spring and oestrus ends in autumn. Oestrus can be provoked by exposure to long photoperiods (such as 14 h light: 10 h dark, LD 14 : 10) during winter² and can be terminated prematurely if short photoperiods (LD 8 : 16) are given during summer³. The pineal is essential for both effects, which are prevented by removal of the gland^{3,4} or its autonomic nerve supply⁵ from the superior cervical ganglia (C.A.Y. unpublished results). The pineal contains large concentrations of the transmitter 5-hydroxytryptamine (5-HT), which has been implicated in the neural control of circadian rhythms, which themselves may be related to the genesis of annual rhythms¹. In the pineal of the rat concentrations of 5-HT vary in phase with photoperiod, and this rhythm is prevented by ganglionectomy⁶. Inhibition of

cerebral 5-HT synthesis prevents circadian rhythms in concentrations of both plasma adrenal corticosteroids⁷ and luteinising hormone⁸. Lesions of the suprachiasmatic nuclei, which contain large concentrations of 5-HT^{9,10}, abolish circadian rhythms in eating and drinking¹¹, in running¹² and in concentrations of pineal *n*-acetyltransferase¹³, which is the rate limiting enzyme in the synthesis of melatonin, a putative pineal hormone. The pineal must transmit information about photoperiod to neural structures regulating the pituitary, but the nature of the link is unknown. We report here that exposure of ferrets to either long or short photoperiods induces differential 5-HT rhythms in the pineal and hypothalamus, which are abolished by autonomic denervation, and that melatonin induces 5-HT rhythms characteristic of animals kept in short photoperiods.

In the first experiment, mature female ferrets were exposed to either long (LD 14 : 10) or short (LD 8 : 16) photoperiods for 8 weeks, the interval required to activate or inhibit oestrus^{3,4}. The ovaries were removed to prevent long days from selectively inducing oestrogen secretion. Illumination was from fluorescent tubes with a spectral output similar to that of daylight; feeding times and temperature fluctuations were similar under long and short photoperiods.

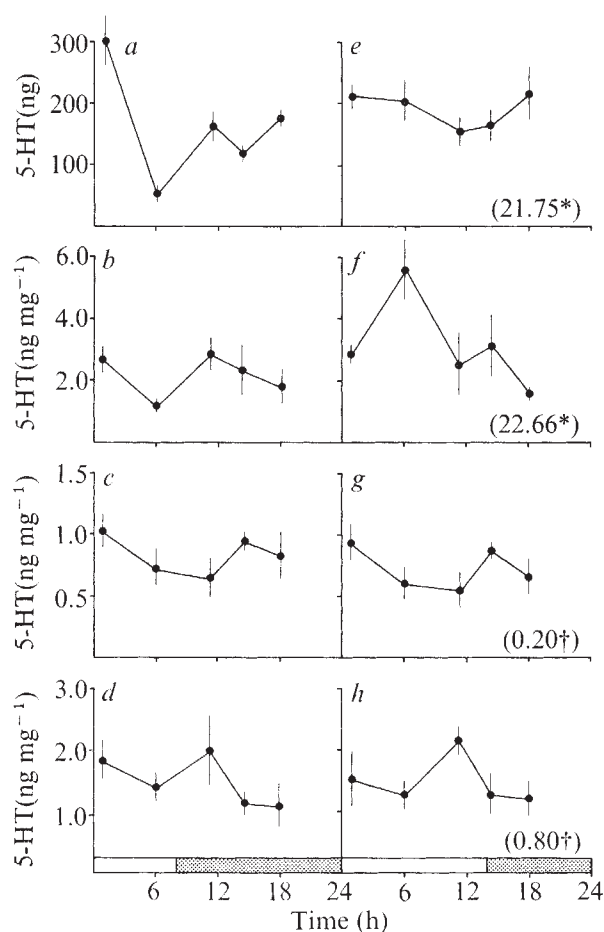
Figure 1 (*a-d*) shows that the circadian rhythm in 5-HT was similar in each brain area examined from animals kept in short photoperiods in spite of considerable differences between them in absolute values. Levels declined during the first 6 h of the photoperiod, rising again during the early part of the dark phase. In long photoperiods, however, the rhythm was different in the pineal and the hypothalamus (Fig. 1*e* and *f*). The rhythm in the pineal was attenuated, and this apparent difference was confirmed by a significant interaction between light treatment and time of day. There was also a striking difference in the 5-HT rhythms in the hypothalamus, levels rising during the first 6 h of the long photoperiod, the opposite of the situation observed during short days.

Circadian changes in 5-HT in midbrain, cerebral cortex and hippocampus appeared to be similar in long and short photoperiods (Fig. 1*g* and *h*) and there was no significant effect attributable to the interaction between the light regimes and time of day in these areas. Thus, two structures (pineal and hypothalamus) known to be concerned with the initiation of oestrus by long photoperiods show distinctive rhythms of 5-HT concentration in this condition, whereas the other areas examined do not.

In the second experiment, the preganglionic fibres to the superior cervical ganglia were severed (decentralised) 5–6 d before the animals were exposed to long or short photoperiods as in the first experiment. Figure 2 shows that circadian 5-HT levels were now similar in both photoperiods, and that those in either the pineal or the hypothalamus no longer exhibited a significant interaction between light treatment and time of day (Fig. 2*c* and *d*). The midbrain, cerebral cortex or hippocampus again showed no differential effects of light, as in the first experiment. The standard deviations of each point in Fig. 2 are conspicuously larger than those in Fig. 1, suggesting that the 5-HT rhythms of decentralised animals might have been "free running", though this conclusion cannot be substantiated on the evidence presented here. Thus, a procedure which prevents the reproductive system of the ferret from responding to either long or short days also prevents the differential effects of the two photoperiods on 5-HT levels in the pineal and the hypothalamus.

Melatonin has been shown to affect cerebral 5-HT levels in rats. Injected 8 h after the onset of a photoperiod (LD 14 : 10), it abolished the circadian rhythm in pineal 5-HT, whereas treatment at 14 h was ineffective¹⁴. It may also increase hypothalamic and midbrain 5-HT levels¹⁵. Melatonin also replicates at least three of the effects of short photoperiods on the ferret's reproductive system, since it terminates the breeding season prematurely³, antagonises the effects of long photoperiods on the initiation of oestrus¹⁶ and restores photosensitivity to ferrets made photorefractory by prolonged exposure to long days³. In

Fig. 1 Circadian rhythms in 5-HT concentrations in various areas of brain, from ferrets kept for 8 weeks in either short (LD 8 : 16) (*a-d*), or long (LD 14 : 10) (*e-f*) days. *a*, *e*, Pineal gland; *b*, *f*, hypothalamus; *c*, *g*, midbrain; *d*, *h*, cerebral cortex. Levels in the hippocampus (not shown) were similar to those in the cerebral cortex. Five or six animals were killed by decapitation 30 min, 6, 11, 14 and 18 h after the lights came on. 5-HT levels in the pineal, whole hypothalamus, midbrain and in samples from ventral hippocampus and dorsal cerebral cortex were measured fluorometrically²². Each point is the mean $\pm$ s.d. in ng per mg wet tissue, except in the pineal (ng per gland) which weighs about 1 mg. The hatched areas signify the dark part of the photoperiod. The figure in parentheses is the *F* ratio of the interaction (light treatment $\times$ time of day) derived from a two-way analysis of variance. **P* < 0.001; †Not significant.

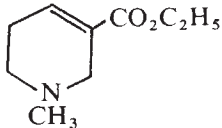
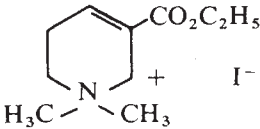
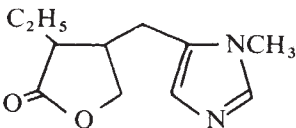
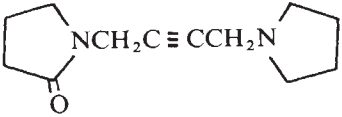
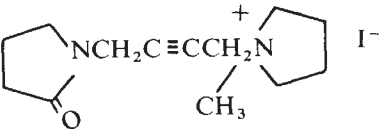
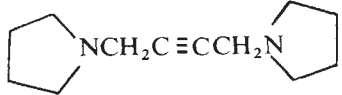
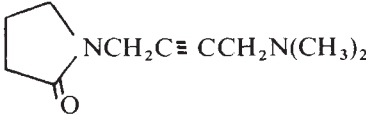
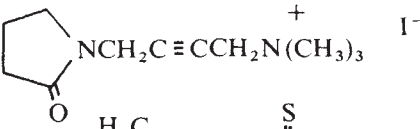
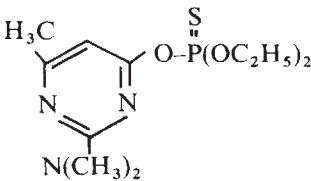
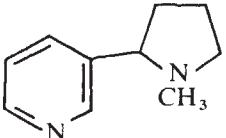


in a solution of the test compound in methanol-acetone (1 : 1), dried on filter paper, and stored in vials for 4 weeks at 25 °C and 85% relative humidity. The concentration of compound required to prevent the production of viable eggs was taken to be the LC_{100} . The results are shown in Table 1, along with those for nicotine and the organophosphorous insecticide pyrimithate (Diothyl, ICI) as references. No significant differences were observed between results for Yeerongpilly and Biarra strains for the cholinomimetics, but pyrimithate was inactive against the latter.

The ixodical activity of the muscarinic agonists examined parallels to a large extent the activity observed in

vertebrate systems. Thus arecoline and pilocarpine have a similar level of activity (on a weight basis) and are considerably less potent than oxotremorine¹⁰. Similarly compound 7, which has 10–20% of the activity of oxotremorine in vertebrate systems^{11,12}, is about 20% as active against ticks. Quaternisation of the nitrogen atom generally caused a marked decrease in ixodical activity, possibly due to poorer penetration, since compound 8, for example, is more active than the tertiary-amino compound (7) in vertebrates^{11,13}, although the methiodides of arecoline and oxotremorine are reported to be less active than the tertiary amines¹⁰. Tremorine, which is oxidised *in vivo* to oxotre-

Table 1 The toxicity of muscarinic agonists to *Boophilus microplus* (Yeerongpilly strain)

Compound no.	Name	Structure	LC_{100} (% $\times 10^2$)
1	Arecoline		50
2	Arecoline methiodide		50
3	Pilocarpine		50
4	Oxotremorine		1
5	Oxotremorine methiodide		50
6	Tremorine		100
7	1-(4-Dimethylaminobut-2-ynyl)pyrrolid-2-one		5
8	1-(4-Dimethylaminobut-2-ynyl)pyrrolid-2-one methiodide		50
9	Pyrimithate		5–10
10	Nicotine		10–20

morine by mammals¹⁴, showed only slight activity against ticks.

It is interesting that oxotremorine is considerably more potent than both nicotine and the reference organophosphorous compound. The cholinomimetics also showed activity against mites, but were completely inactive against *Musca domestica* (adults), *Aedes aegypti* (larvae), and *Lucilia pericata* (larvae). Further work is required to determine whether this difference between Insecta and Acarina is morphological or biochemical in origin.

In conclusion, the muscarinic agonists examined are acaricidal, and in some cases are highly potent. We suggest that this unexploited biochemical lesion may provide a useful starting point for the design of new acaricides.

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Transmission without spikes between locust interneurons and motoneurons

MANY neurones, particularly in sensory systems of both vertebrates and invertebrates, do not produce action potentials¹⁻³. In motor pathways within the central nervous system of arthropods there are interneurons whose slow changes in membrane potential can affect the frequency of action potentials in motoneurons⁴⁻⁶. Some of these interneurons normally produce spikes⁶, but others apparently do not^{4,5}. We have studied the properties of synaptic transmission between these non-spiking interneurons and motoneurons in the locust, *Schistocera gregaria*, in the course of investigating the integrative mechanisms underlying the generation of motor patterns⁷.

Intracellular recordings were made in the metathoracic ganglion⁸ from dorsally located somata of hindwing and hindleg motoneurons, identified physiologically by simultaneous extracellular recordings from appropriate muscles. Each motoneuron was individually identified so that intracellular recordings could be made from the same neurone in different locusts. Non-spiking neurones were penetrated intracellularly in the dorsal neuropile close to the neuropilar processes of the motoneurons. Most recordings were made with electrodes filled with potassium acetate but some were made with electrodes filled with cobaltous chloride⁹ to allow the subsequent staining of the motoneurons and non-spiking neurones (Fig. 1). The non-spiking neurones have profuse arborisations within the metathoracic ganglion, but have no projections to other ganglia, nor any processes emerging in peripheral nerves; hence they are intraganglionic interneurons.

In a quiescent locust these interneurons are modulated

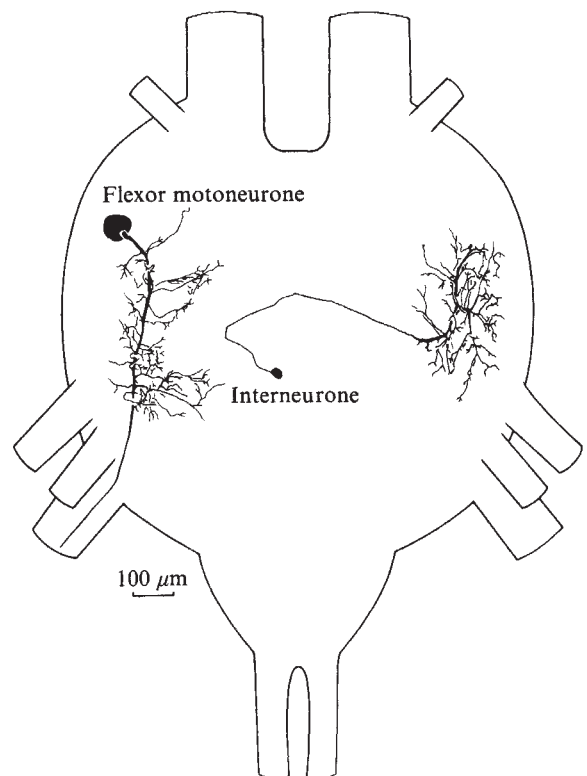


Fig. 1 Drawings of an interneurone and a motoneurone stained by intracellular injection of cobalt as viewed dorsally in a whole mount preparation of the metathoracic ganglion. The neurones were filled on the same side in different locusts but for clarity are drawn on opposite sides of the same ganglion. Left, the lateral fast flexor motoneurone of the left hind tibia whose soma is on the dorsal surface of the ganglion; right, an interneurone whose soma is ventral, which when depolarised resulted in a depolarisation of this flexor motoneurone. The extensive arborisations of the two neurones overlap in all three dimensions.

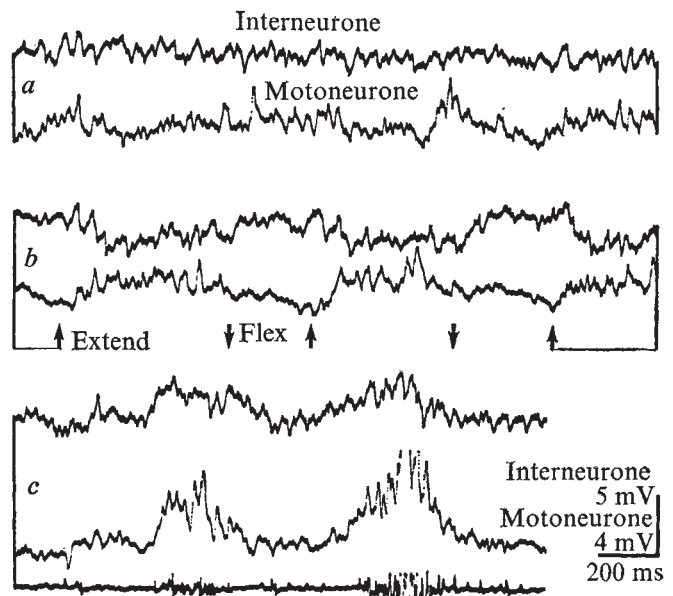


Fig. 2 Spontaneous and evoked sequences of synaptic potentials in a fast motoneurone innervating the flexor tibiae of the hind leg (lower traces) and in a presynaptic non-spiking interneurone (upper traces). *a*, In a quiescent locust both the interneurone and the motoneurone are modulated by a steady stream of synaptic potentials. *b*, Imposed extensions of the tibia of the hind leg, beginning at the upward arrows, result in a depolarisation of the motoneurone and a hyperpolarisation of the interneurone. *c*, Flexions of the tibia evoked by touching the head are accompanied by a depolarisation of the interneurone. Potentials of the flexor tibiae muscle are monitored extracellularly on the third trace.

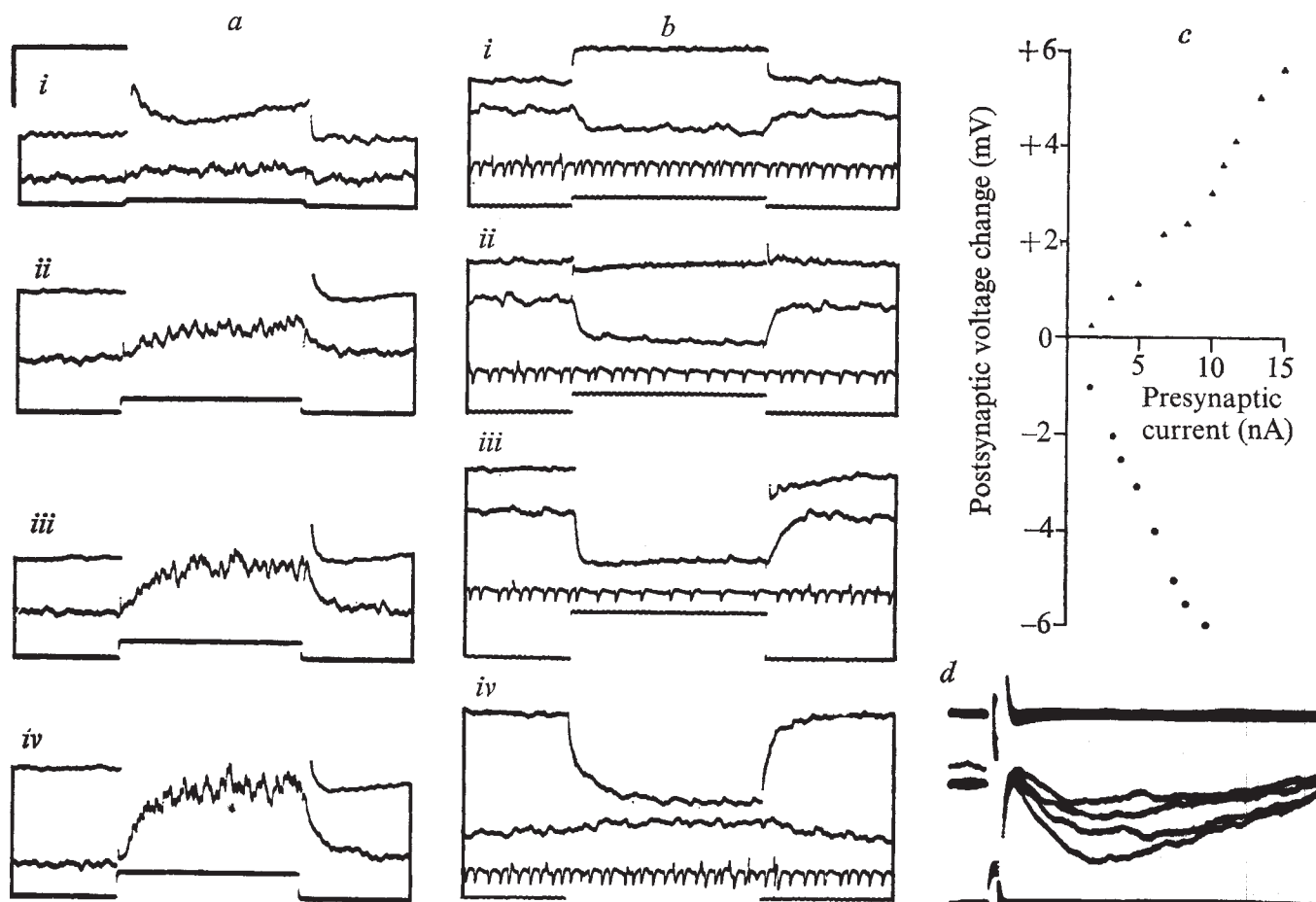
by a steady stream of synaptic potentials, but do not spike (Fig. 2a). Imposed or evoked movements of the joint whose motor units they affect result in changes in the membrane potential of the interneurone (Fig. 2b and c). The interneurons do not spike, either as a result of synaptically driven depolarisations, or in response to an injected depolarising current as high as 50 nA. This current strength is at least ten times that needed to evoke spikes when injected into the neuropilar segment of a motoneurone. Currents as low as 2 nA injected into an interneurone result in a change in the membrane potential of motoneurons.

We have observed three effects on motoneurons of passing current into interneurons: first, an applied depolarisation of an interneurone results in the depolarisation of a motoneurone (Fig. 3a, i-iv); second, a depolarisation results in the hyperpolarisation of a motoneurone (Fig. 3b, i-iii); third, in some interneurons that produce the second effect, hyperpolarisation results in the depolarisation of the motoneurone (Fig. 3b, iv). Single interneurons have postsynaptic effects upon more than one motoneurone, as revealed by changes in the frequency of extracellularly recorded poten-

tials in muscles innervated by other motoneurons (Fig. 3b).

The following analysis is concerned with the properties of transmission between interneurons and motoneurons when an interneurone is depolarised. Transmission is graded. A pulse of current lasting a few hundred ms injected into an interneurone results in a slow change in the membrane potential of the motoneurone. There is no effect on the motoneurone if the same amount of current is passed when the electrode is just outside the interneurone. With intracellular stimulation of the interneurone, the amplitude of the slow change is linearly related to the magnitude of the depolarising current over the range tested (Fig. 3c). At higher current strengths the postsynaptic depolarisations can be large enough to evoke action potentials in the motoneurons. Brief pulses injected into an interneurone result in changes in the membrane potential of the motoneurone, which vary continuously with the strength of the applied current (Fig. 3d). At no time during brief stimulation of the interneurone does the membrane potential of the motoneurone change abruptly as would be expected if the interneurone itself, or an intervening interneurone, had spiked.

Fig. 3 Effects of non-spiking interneurons (first traces) upon a fast motoneurone (second traces) innervating a bifunctional muscle, the tergotrochanteral. This muscle elevates the hind wing in flight and depresses the trochanter of the hind leg in walking. *a*, *i-iv*, An interneurone which when depolarised results in a graded depolarisation of the motoneurone in a ganglion with all peripheral nerves cut. Current (third traces) was injected through the high resistance recording electrodes by means of a bridge circuit which was out of balance except at the lowest current strengths. *b*, A second interneurone in another locust affecting the same tergotrochanteral motoneurone: *i-iii*, depolarisation results in a graded hyperpolarisation of the motoneurone; *iv*, hyperpolarisation of the interneurone results in a slight depolarisation of the motoneurone which presumably indicates a decreased rate of release of transmitter. The third traces monitor the extracellular potentials of some coxal muscles of the hind leg whose motoneurons are also affected by this interneurone; the downward deflections are from the anterior adductor of the coxa, whose potentials are reduced in frequency when the interneurone is depolarised; the upward deflections are from another coxal muscle that is excited when the interneurone is hyperpolarised. The fourth traces monitor current. *c*, The graded nature of the transmission between the interneurons and the tergotrochanteral motoneurone is indicated by the relationship between presynaptic current and the change in postsynaptic voltage. Triangles are measurements taken from records of the interneurone-motoneurone pair shown in *a*; circles are from the pair shown in *b*. The voltage of the motoneurone was measured 300 ms after the onset of the stimulus. *d*, 1.5-ms current pulses of increasing strengths injected into the interneurone shown in *b* result in graded changes in voltage in the motoneurone. Calibration—Voltage: *a*, motoneurone 3.8 mV, interneurone 20 mV; *b*, motoneurone 7.7 mV, interneurone 20 mV; *d*, motoneurone 1.5 mV; interneurone 15 mV; current: *a*, 33 nA; *b*, 17 nA; *c*, 75 nA. Horizontal: *a*, *b*, 400 ms; *d*, 14.5 ms.



The most economical interpretation of the graded effect is that the interneurons synapse directly upon the motoneurons. The synaptic delay cannot be measured accurately because transmission is graded. Pulses (1 ms) of current injected into an interneurone are followed within 1 ms by a voltage change in the motoneurone, a delay similar to that observed between spiking interneurons and leg motoneurons of the locust. The gross anatomy is consistent with a monosynaptic connection because there is considerable spatial overlap in the neuropile of the fine branches of an interneurone and a motoneurone upon which it exerts an effect (Fig. 1).

The connections between the interneurons and motoneurons that we have encountered so far, seem to be chemically and not electrically mediated, for the following reasons. First, current of either polarity injected into a motoneurone has no effect upon the membrane potential of the presynaptic interneurone. Spikes in the motoneurone are not reflected in the interneurone. Second, a brief square wave depolarising pulse injected into an interneurone decays rapidly, but evokes a potential in the motoneurone that can persist for 45 ms (Fig. 3d). This prolonged effect is probably the result of transmitter release from the interneurone. It is not the result of passively charging the motoneurone through an electrical synapse, because the postsynaptic potential reaches its peak some 10 ms after the end of the stimulus, and is asymmetrical. Third, in those interneurons where depolarisation results in the hyperpolarisation of a motoneurone, the reversal of sign is most easily explained by supposing that the depolarisation increases the rate of release of a transmitter substance that has an inhibitory effect upon the motoneurone. Fourth, there is often a progressive increase in the transmembrane voltage fluctuations recorded in a motoneurone as it is depolarised by increasing amounts of current injected into an interneurone (Fig. 3a). Increased fluctuations of voltage have not been observed when a motoneurone is hyperpolarised by depolarisation of an interneurone. This is a consistent observation but we have no evidence which would favour one of many possible explanations. Current injected directly into the motoneurone, causing a similar shift of its membrane potential, does not give rise to an increase in the fluctuations of the transmembrane potential. The increased fluctuations in voltage on depolarisation can therefore be attributed to synaptic transmission. They are not caused by spikes in sensory feedback loops, as they are still recorded in a ganglion with all its peripheral nerves cut. There remain two possible explanations as to their origin. First, the interneurone has a direct connection with the motoneurone, but also excites spiking interneurons that in turn synapse on the motoneurone. This possibility of parallel pathways cannot yet be eliminated. Second, the voltage fluctuations could result from the release of transmitter from the interneurone directly on the motoneurone. This may be the only mechanism or it may operate in conjunction with the first mechanism. The effect is similar to that seen in a frog muscle fibre when the terminals of its motoneurone are depolarised by pulses of current which do not evoke action potentials⁹. At the frog neuromuscular junction the voltage fluctuations result from the random release of transmitter substance and a similar explanation may be appropriate for the non-spiking intraganglionic interneurons modulate the membrane potential of motoneurons by graded release of transmitter through monosynaptic pathways.

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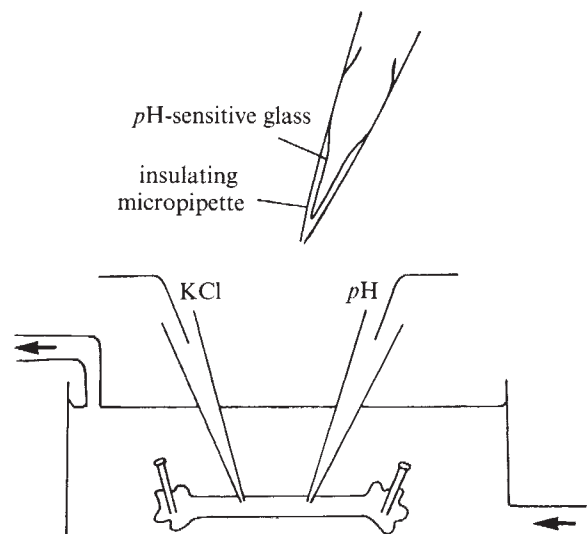
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Microelectrode measurement of the intracellular pH of mammalian heart cells

RECESSED-TIP pH-sensitive microelectrodes¹ can be made sharp enough to be used on mammalian muscle cells. They permit not only the direct measurement of the intracellular pH (pH_i), but also the continuous recording of changes in pH_i during experiments lasting for several hours. We report here the first measurements of the pH_i of mammalian cardiac tissue made with this type of pH-sensitive microelectrode. Apart from one report of experiments with an open-tipped microelectrode², the only previous determinations of cardiac pH_i have been made by the CO_2 or DMO methods³, which are indirect and can detect only very slow changes. Earlier designs of pH-sensitive glass microelectrodes have been of little use because of their large size. The recessed-tip design (Fig. 1) lacks this drawback, as only its extreme tip, which can be as small as 0.5 μm in diameter, needs to be inside the cell. We have used these electrodes successfully in ventricular muscle preparations from several animals, but the results described here are confined to those obtained with sheep heart Purkinje fibres.

Our experimental arrangement is illustrated in Fig. 1. Free-running single Purkinje fibres were cut from sheep ventricles. A little side wall material was left attached to pin the fibre in the perfusion chamber where it was superfused at 35 °C. A recording from an experiment with this tissue is shown in Fig. 2. The pH-sensitive microelectrode recorded the membrane potential of a Purkinje fibre cell, minus an offset voltage proportional to pH_i , while a neighbouring cell was impaled with the KCl microelectrode. The KCl microelectrode output voltage was subtracted electronically from that of the pH electrode so that when both electrodes were intracellular, a pH_i recording could

Fig. 1 Diagrammatic illustration of the preparation pinned into the experimental chamber. It was superfused continuously, and impaled with a conventional, KCl-filled microelectrode (left), and the pH-sensitive microelectrode (right). A diagram (not to scale) of ~ 0.2 mm of the tip of the latter is shown at the top.



be obtained (Fig. 2, lower trace) which was independent of any changes in membrane potential. (Ideally both electrodes should be in the same cell. This is theoretically possible, but in practice it was found difficult to obtain good penetrations with both electrodes when the interelectrode distance was $< 100 \mu\text{m}$.) In this experiment, the preparation was perfused initially with saline equilibrated with 5% CO_2 , 95% O_2 . External CO_2 was then removed by changing the perfusion solution to one equilibrated with 100% O_2 . This produced a transient increase in $p\text{H}_i$ followed by a partial recovery to a level higher than in the absence of CO_2 . On return to 5% CO_2 there was a transient $p\text{H}_i$ decrease followed by a return to the control level. Essentially similar recoveries from changes in $p\text{H}_i$ when

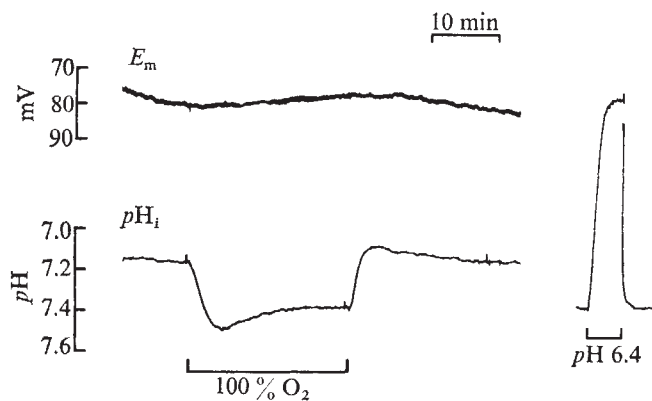


Fig. 2 Pen recording of an experiment to measure $p\text{H}_i$ and the response to removal and replacement of external CO_2 . The membrane potential was recorded by a conventional microelectrode (top trace) and the $p\text{H}_i$ obtained when the $p\text{H}$ -sensitive electrode is also intracellular (bottom trace). The $p\text{H}$ -sensitivity of the electrode was tested (right) by a reduction of the solution $p\text{H}$ to 6.40 (both electrodes extracellular). The preparations were perfused with a bicarbonate-buffered modified Tyrode solution composed of 140 mM Na^+ , 4 mM K^+ , 2 mM Ca^{2+} , 1 mM Mg^{2+} , 2 mM pyruvate, 10 mM glucose, chloride and bicarbonate (reciprocally varied) to give a $p\text{H}$ of 7.40 at 35°C . The bicarbonate required was determined experimentally by titration. CO_2 levels were determined by a CO_2 gas analyser. These varied in nominally 95% O_2 : 5% CO_2 gas cylinders from 4.25 to 4.79% CO_2 (mean 4.61). Thus the bicarbonate concentration ranged from 17.5 to 22 mM. In the absence of CO_2 and bicarbonate, solutions contained 10 mM HEPES (*N*-2-hydroxyethyl piperazine-*N*-2-ethanesulphonic acid) buffer (also at $p\text{H}$ 7.40). The changes in the $p\text{H}_i$ when CO_2 was removed were not altered when Tris-maleate buffer was substituted for HEPES.

CO_2 was changed have been observed in crab muscle⁴, squid axon⁵ and snail neurones⁶.

The results from a total of 14 experiments are summarised in Table 1. The mean $p\text{H}_i$ in the absence of CO_2 is close to 7.2 and is significantly higher than when CO_2 is present. In general, our values for $p\text{H}_i$ are somewhat higher than those obtained previously using the DMO technique in dog heart: 6.77 (ref. 7), 6.86 (ref. 8), and 7.01 (ref. 9) although Benson *et al.*¹⁰ have reported similar values to those we have found. Lavallée² used

Table 1 Intracellular $p\text{H}$ and membrane potential

Equilibration gas	$p\text{H}_i$	E_m (mV)
100% O_2	7.20 ± 0.05	71.8 ± 3.7
95% O_2 : 5% CO_2	7.02 ± 0.04	78.1 ± 2.5

Results are means $\pm$ s.e.m.; $n = 14$. Those from preparations with a membrane potential in 5% CO_2 of < 60 mV were rejected.

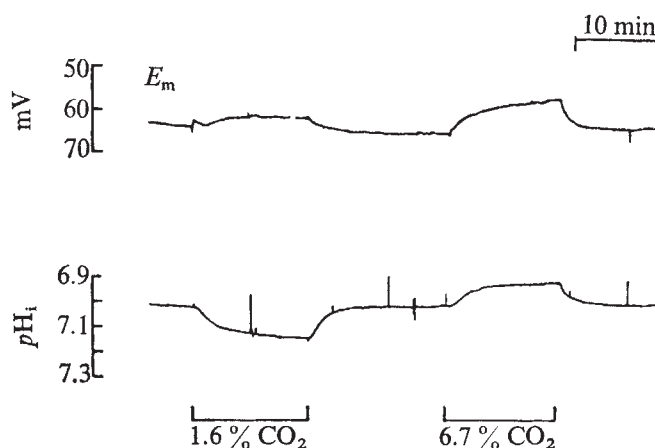


Fig. 3 Change in membrane potential (top) and $p\text{H}_i$ (bottom) when the external CO_2 level was changed from 4.7% to the levels indicated. The bicarbonate concentration (20.5 mM) was maintained constant so that the external $p\text{H}$ varied from 7.40 in the control solution to 7.70 and 7.19 respectively in the two test solutions. The potassium concentration in this experiment was 8 mM.

open-tipped $p\text{H}$ -selective microelectrodes and found a $p\text{H}_i$ of 6.9 in rat atrial fibres (stimulated at a rate of 200 min^{-1}).

From experiments of the type illustrated in Fig. 1 it is clear that $p\text{H}_i$ is greatly influenced by CO_2 in the equilibration gas. We have used different CO_2 : O_2 mixtures to see how $p\text{H}_i$ varies with alteration of the external $p\text{H}$, and the results of an experiment of this type are illustrated in Fig. 3. All solutions contained the same bicarbonate concentration and were equilibrated with the CO_2 levels indicated. (The $p\text{H}$ of these mixtures being measured for each experiment.) Alteration of the CO_2 content produced a change in $p\text{H}_i$ to a new steady level within ~ 10 min, presumably by the reaction



A linear relationship was found between $p\text{H}_i$ and the external $p\text{H}$, but the $p\text{H}_i$ change was only ~ 40 per cent of the change in external $p\text{H}$. This difference is caused by intracellular buffering.

These responses differ from those observed when the bicarbonate concentration is altered as well as the CO_2 content to maintain a constant external $p\text{H}$. In this case, the responses are similar to those shown in Fig. 1, that is, a large transient initial $p\text{H}_i$ change followed by a partial recovery to a level nearer that of the control.

Direct measurement of $p\text{H}_i$ with recessed-tip $p\text{H}$ sensitive microelectrodes thus enables continuous recording of previously undetectable transient changes. The results show that alteration of CO_2 levels produces rapid changes in $p\text{H}_i$. The partial recovery of these changes when external $p\text{H}$ is unchanged suggests the presence of some sort of H^+ pump in the cell membrane.

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Origin of plasma α_2 HS-glycoprotein and its accumulation in bone

WE have shown previously that a glycoprotein constituent of calcified cortical bone matrix is immunochemically identical to a component of blood plasma^{1,2}. In bone and dentine this specific α -glycoprotein is concentrated in amounts relative to plasma albumin but is not present at greater concentrations than in plasma in various other tissues. This material, although mainly present in extravascular sites in bone, could originate from the plasma because it represents a substantial proportion of the radioactivity in bone tissue 12 d after injection of ^{14}C -total plasma glycoprotein¹. Rabbit bone α -glycoprotein is analogous to human α_2 HS-glycoprotein^{3,4}, which is located in mineralising areas in normal human bone matrix⁵. To our knowledge direct conclusive proof of the tissue origin of the plasma α_2 HS-glycoprotein is lacking although most of the serum glycoproteins seem to be synthesised by the liver^{6,7}. Nevertheless, its increased concentration in calcified tissues suggests that its presence in plasma could be a result of synthesis and release by bone tissue. We now report that the α_2 HS-glycoprotein is synthesised by the liver and a

Fig. 1 Total radioactivity in liver perfusion medium (solid line), and the proportion precipitable by TCA (dashed line), with time after introduction of ^{14}C -glucosamine into the perfusate. The perfusion medium consisted of 2.6 g % (w/v) bovine plasma albumin in bicarbonate buffer⁹ with bicarbonate added to 25 mM concentration and the addition of human erythrocytes to give 2.5% (w/v) haemoglobin. Blood contained in the liver was washed out with 30 ml perfusion medium before addition of 20 μCi D-1- ^{14}C -glucosamine (The Radiochemical Centre, Amersham) to the perfusate. Perfusion was with a total of 50 ml of this medium at a flow rate of 27 ml min⁻¹. Samples (0.5–1 ml) of perfusate were taken at various time intervals up to 4 h after the addition of D-1- ^{14}C -glucosamine and centrifuged at 2,000g for 30 min at 4 °C to remove erythrocytes. Portions (10 μl) of the supernatants were dissolved in 0.5 ml solute (Packard) and 5 ml scintillation fluid¹⁰ was added for assay of radioactivity by liquid scintillation. Samples (0.1 ml) of the supernatants were added to 1 ml 12% (w/v) aqueous TCA and allowed to stand overnight at 4 °C. The TCA precipitates were collected by centrifugation at 4 °C at 2,000g for 15 min and washed three times with 1 ml 12% (w/v) aqueous TCA containing 1 mg glucosamine ml⁻¹. The washed precipitates and individual supernatants were dissolved in solute (1 ml) and their radioactive contents determined. All radioactivity determinations were corrected for quenching by internal standardisation.

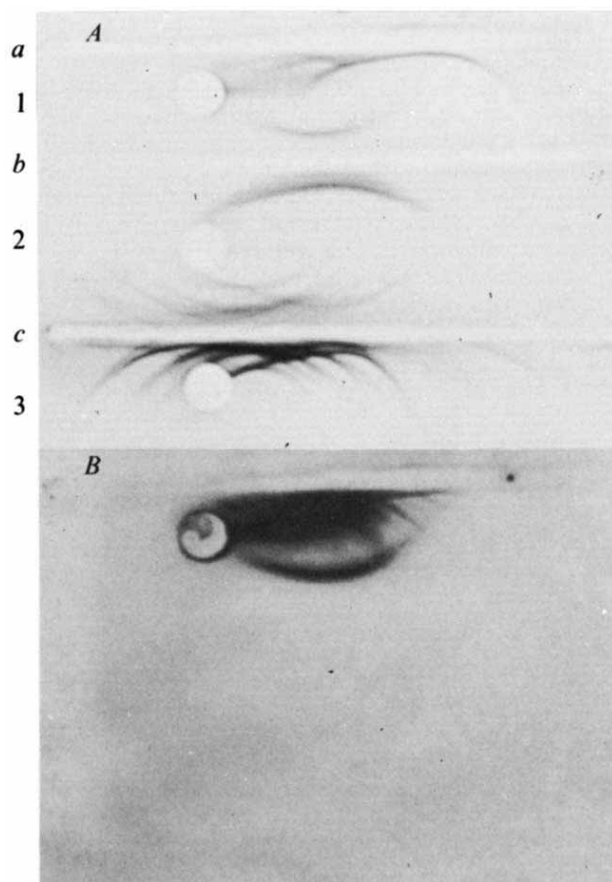
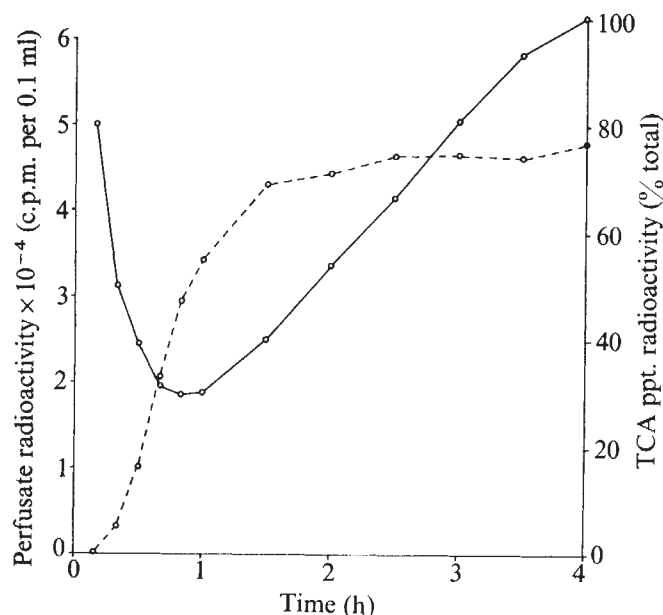


Fig. 2 Immunoelectrophoresis in 1% (w/v) agar gel in sodium barbitone buffer, pH 8.6, of rabbit liver perfusion medium and rabbit plasma against antiserum to rabbit plasma and antiserum to rabbit bone α -glycoprotein (A) and its autoradiograph (B). Electrophoresis was for 2 h at 100 V and autoradiography was by exposure to Kodirex X-ray plates (Kodak) for 10 d. Well (1), Perfusion medium 4 h after adding D-1- ^{14}C -glucosamine. Well (2), Rabbit plasma to which an amount of D-1- ^{14}C -glucosamine was added which was equivalent to the radioactivity present in well (1). Well (3), Rabbit plasma, non-radioactive. Troughs (a) and (c), Anti-rabbit plasma antiserum. Trough (b), Anti-rabbit bone α -glycoprotein antiserum.

proportion is subsequently accumulated in bone tissue.

Livers of 300–350 g Dutch rabbits were perfused *in situ* with medium containing D-1- ^{14}C -glucosamine by a modification of the method of Hems *et al.*⁸. Total perfusion medium radioactivity fell rapidly to about the 1 h time point as the perfused liver removed radioactive precursor. This value then increased for the rest of the perfusion period (Fig. 1). With time of perfusion the radioactivity which was precipitable by trichloroacetic acid (TCA) increased from 5% at 20 min to 77% at 4 h. Column chromatography on BioGel P30 confirmed that TCA precipitability represented macromolecular radioactivity as similar results were obtained by both methods. Macromolecular radioactivity first appeared in the medium 10–20 min after addition of labelled precursor and this indicates the time required for uptake, synthesis and secretion of the glycoproteins by the liver¹¹. 7% (w/v) polyacrylamide disk gel electrophoresis of the perfusion medium showed that a large proportion of the radioactively-labelled material had α -electrophoretic mobility (not shown).

Agar gel immunoelectrophoresis followed by autoradiography (Fig. 2) indicated that the liver perfusate contained numerous proteins reactive against polyvalent anti-rabbit plasma antiserum and their synthesis during perfusion is demonstrated by the autoradiogram. Antiserum against

purified rabbit bone α -glycoprotein was produced as described previously¹ and Fig. 2 shows that liver synthesised a component showing immunological identity with this glycoprotein. No interaction between the immunoprecipitates and D-1-¹⁴C-glucosamine during the washing and staining procedure was observed (Fig. 2). These results show that the liver is capable of synthesising the α -glycoprotein which has been found to make up approximately 4% of the rabbit cortical bone non-collagenous organic matrix¹.

To test whether bone tissue could also synthesise this component, eight rabbit half-calvaria (25 d gestation) were cultured, each in 1.5 ml medium for 48 h in the presence of 7.5 μ Ci 1-¹⁴C-glucosamine, by the method of Reynolds¹². After culture the embryonic bones were individually pooled and the non-collagenous proteins prepared by the method of Herring *et al.*¹³.

The culture medium at 48 h contained very little radioactivity in macromolecular components (0.6% of total medium radioactivity) as assessed by chromatography on BioGel P30. DEAE-cellulose chromatography of macromolecular radioactivity from cultured bone collagenase digests showed that only 17% of the total radioactivity applied was eluted in the position of the particular α -glycoprotein under investigation^{1,2}. This contrasts with the situation *in vivo* after D-1-¹⁴C-glucosamine injection where a greater proportion is eluted in this less acidic fraction¹.

Immunoelectrophoresis of the less acidic fractions of

the rabbit α -glycoprotein analogous to human α_2 HS-glycoprotein, which is concentrated in bone tissue relative to plasma albumin, is synthesised by the liver. These results are also the first direct demonstration that the α_2 HS-glycoprotein is made by the liver, as implied by the work of Miller^{6,7}.

The accumulation of this α -glycoprotein in calcified matrix could be a result of its affinity for adsorption sites on bone mineral and our current work³ confirms that this concentration effect occurs during the precipitation of calcium phosphates *in vitro*. This suggests a relatively high affinity of this plasma protein for calcium and the concentration in bone matrix could represent an aspect of a more basic involvement in calcium metabolism.

Certain serum fractions have been shown to delay the rate of transformation *in vitro* of an amorphous calcium phosphate to a more crystalline hydroxyapatite¹⁴. Whether the binding of the α -glycoprotein has any specific influence upon the rate of formation or on the nature of the bone mineral phase *in vivo* is not known. Whatever the physiological function of the particular affinity of the α_2 HS-glycoprotein for bone matrix, our demonstration that this material is synthesised by the liver but not by bone tissue has interesting implications for bone physiology.

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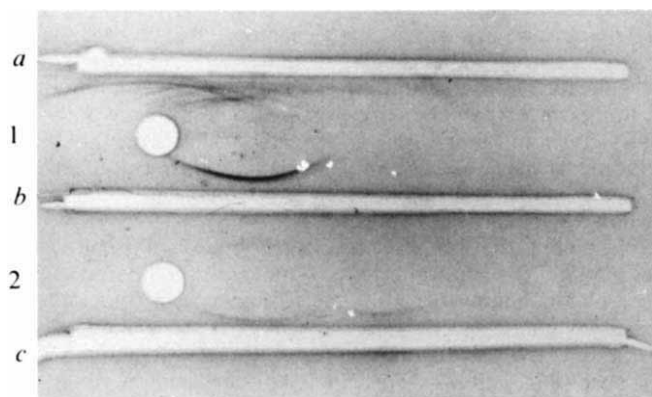


Fig. 3 Bone non-collagenous proteins prepared by the method of Herring *et al.*¹³ were chromatographed on DEAE-cellulose as described previously^{1,2}. The peak of radioactivity corresponding to the 0-0.2 M NaCl eluate was pooled, dialysed exhaustively against distilled water and freeze dried. The freeze-dried material was dissolved in 0.05 M Tris-HCl buffer pH 7.2 (0.2 ml) and samples (10 μ l) of the solution used for immunoelectrophoresis in agar gel (1% w/v in sodium barbitone buffer pH 8.6). Stained with 1% amido black in 7% acetic acid. Well (1), 0-0.2 M NaCl eluate + normal rabbit plasma. Well (2), 0-0.2 M NaCl eluate. Troughs (a) and (c), Anti-rabbit plasma antiserum (40 μ l). Trough (b), Anti-rabbit bone α -glycoprotein antiserum (40 μ l).

cultured bone collagenase digests, which contained appreciable amounts of radioactivity, showed detectable amounts of α -glycoprotein (Fig. 3). But neither this immunoprecipitate nor any serum protein precipitated by polyvalent anti-rabbit plasma antiserum (Fig. 3) contained radioactivity as assessed by autoradiography on Kodirex X-ray plates for up to 60 d. Therefore embryonic bone tissue cannot synthesise this particular α -glycoprotein, even though it is present in foetal plasma (our unpublished observations).

The present results confirm our previous suggestion¹ that

Hybrid molecules and the superiority of the heterozygote

POPULATION geneticists have never agreed on the extent to which overdominance of fitness—that is, the situation in

Table 1 Comparisons of heterozygosities at monomeric and multimeric enzyme loci of four animal and two plant species*

Species	No. of monomeric enzymes	No. of multimeric enzymes	Normal deviate	P	Source
<i>Homo sapiens</i>	20	35	-1.369	0.085	Table 2
<i>Peromyscus polionotus</i> †	4	15	-2.160	0.015	21
<i>Astyanax mexicanus</i> ‡	5	11	-2.553	0.005	22
<i>Drosophila willistoni</i> §	16	15	-1.622	0.052	23
<i>Stephanomeria exigua</i> ssp. <i>carotifera</i> ¶	3	5	-2.007	0.018	24
<i>Lupinus texensis</i> ††	4	3	-1.591	0.056	25

*For *Homo sapiens* heterozygosities (H) were obtained directly from the source; for the other species they were calculated as $H = 1 - \sum p_i^2$ where p_i is the frequency of the electrophoretic allele weighted over populations. Heterozygosities at the two classes of enzymes were compared by applying the Wilcoxon two-sample rank test corrected for ties and discontinuity⁶. Symbolism of enzymes as in source.

†Monomers: Est-1 and 2, PGM-1 and 3; multimers: Est-3 and 4, PGI-1, LDH-1, LDH-2, LDH-3, MDH-1, MDH-2, MDH-3, α -GPDH-1, 6-PGDH-1, GOT-1, GOT-2, IDH-1, IDH-2. Amount of variation or degree of polymerisation unknown for the other enzyme loci listed in Selander *et al.*²¹.

‡Monomers: Pep-1, PGM-1, Est-1, 2 and 3; multimers: α GPD-1, GPGD-1, PGI-1, PGI-2, GOT-1, GOT-2, MDH-1, MDH-2, IDH-2, LDH-1, LDH-2.

§Monomers: Lap-5, Est-2, 3, 4, 5 and 7, ALD-1 and 2, G6PDH, ME-2, PGM-1, ADK-1 and 2, HK-1, 2 and 3; multimers: ACPH-1, ADH, MDH-2, α GPDH, Fum, GOT, HBDH, IDH, ODH-1, ME-1, XDH, AO-1, AO-2, TO, TPI-2.

¶Monomers: Est-2 and 3, APH-1; multimers: GOT-1, 2 and 3, PGI, TO-1.

††Monomers: Est-1, Lap-1, PGM-1, ACPH-1; multimers: PGI-1, GOT-1 and 2.

Table 2 Heterozygosities (H) at 50 human enzyme loci*

Multimers	H	Source
Phosphohexose isomerase	0.0006	7
Lactate DH A	0.0020	7
Lactate DH B	0	7
NADH-isocitric DH† (soluble)	0.0056	7
NADH-isocitric DH (mitochondrial)	0	7
NAD-malate DH (soluble)	0.0019	7
Phosphogluconate DH	0.0372	7
Alcohol DH 1	0	7
Alcohol DH 2	0.0582	7
Alcohol DH 3	0.4824	7
Superoxide dismutase A	0.0006	7
Glutamate oxaloacetate transaminase (soluble)	0.0017	7
Glutamate oxaloacetate transaminase (mitochondrial)	0.0334	7
Glutamic-pyruvic transaminase (soluble)	0.4950	7
Nucleoside phosphorylase	0.0013	7
Acid phosphatase 2	0	7
Acid phosphatase 3	0.0011	7
Pyrophosphatase	0	7
Alkaline phosphatase (placental)	0.5020	7
Peptidase A	0.0009	7
Peptidase D	0.0140	7
Esterase D	0.1752	7
Glycerol-3-phosphate DH 2	0.0014	9
Malic enzyme (mitochondrial)	0.3000	8
Malic enzyme (soluble)	0	8
Glutathione peroxidase	0.0077	10
Phosphoglyceric acid mutase	0.0019	11
Glyoxalase I (red cells)	0.4771	12
NAD-malate DH (mitochondrial)	0.0004	13
2-3-Diphospho-glycerate mutase	0	8
Sorbitol DH	0	8
Cytidine deaminase	0.4545	14
Monomers	H	Source
Phosphoglucomutase 1	0.3664	7
Phosphoglucomutase 2	0.0007	7
Phosphoglucomutase 3	0.3828	7
NADH diaphorase	0.0101	7
Adenylate kinase	0.0768	7
Adenosine diaminase	0.1004	7
Acid phosphatase 1	0.5190	7
Peptidase B	0.0021	7
Peptidase C	0.0062	7
Fucosidase	0.3720	15
Hexokinase III	0.0303	16
Aconitase (soluble)	0.0159	17
Aconitase (mitochondrial)	0	17
Carbonic anhydrase CA _{II}	0	18
Amylase (pancreatic)	0.0825	19
Amylase (salivary)	0.0141	20
Acetylcholinesterase	0	8

*Data refer to caucasians only. No variants were detected at other enzyme loci listed in Harris *et al.*⁷ and in Harris and Hopkinson⁸, or there is no direct information as to whether they are monomers or multimers. In loci with very low heterozygosities observed differences in H may be of no significance. Loci with an H less than 0.01 may be characterised as monomorphic. Using this criterion 22 out of 33 multimers and 6 out of 17 monomers are monomorphic ($\chi^2 = 4.48$, d.f. = 1, $P = 0.034$). †DH, dehydrogenase.

which the fitness of the heterozygote exceeds the fitness of both homozygotes—is responsible for the maintenance of genetic variability in populations¹. It has been proposed²⁻⁴ that heteromultimers formed by random association of enzyme subunits coded by two different alleles provide a molecular basis for overdominance. This hypothesis stems from the observation that heteromultimers often differ from homomultimers in a number of aspects⁵, sometimes restoring enzymatic activity when both homozygotes lacked such activity². Clearly, the crucial step towards associating (or disassociating) observations of this type with overdominance of fitness must be taken if we were to test the hypothesis that heteromultimers *per se* are responsible for the high amounts of variation observed in populations. I argue here that the enormous amounts of electrophoretic data now available exclude this hypothesis.

In Table 1 the amounts of electrophoretic variability of loci coding for multimeric enzymes are compared with those of loci coding for monomeric enzymes in four animal and two plant species. In all cases heterozygosities at multimers are lower than heterozygosities at monomers. The species in Table 1 were selected either because a reasonably large number of both types of enzyme loci were studied and because the number of assayed individuals is quite high to allow one to ignore errors in gene frequency estimates, or because they represent broad taxonomic units embracing, practically, all diploid organisms. Data from other species of mammals, lizards, fish, fruit flies, and snails could be cited, the great majority of which seem to support this conclusion (for data on human enzyme loci see Table 2).

It has been argued that enzymes with multiple substrates⁶ and enzymes regulating the flux through a pathway⁷ are more variable than enzymes with single substrates or non-regulatory enzymes. The class of non-regulatory enzymes contains more multimers than monomers so it can be argued that the difference in amounts of variation is ultimately related to function rather than to presence or absence of hybrid bands. The validity of these hypotheses is in doubt as there is a growing list of enzymes with multiple substrate and with regulatory properties which, nevertheless, are exceptionally conservative²⁸. But the argument that the difference in amount of polymorphism indicated here is ultimately related to function rather than to degree of polymerisation can be dealt with in a more direct way. In Table 3 I have listed amounts of electrophoretic variability of monomeric and multimeric enzymes of same *in vitro* biochemical function. Out of 27 pair-wise comparisons that

Table 3 Heterozygosities at monomeric and multimeric enzymes having the same *in vitro* function

Species	Monomer	Multimer	Source
<i>D. subobscura</i>	Est-3 0.448 Est-5 0.549	Est-7 0.168	29
<i>D. mojavensis B</i>	Est-2 0.081	Est-4 0.551 Est-5 0.565	30
<i>D. arizonensis</i>	Est-2 0.403	Est-4 0.573 Est-5 0.723	30
<i>D. mulleri</i>	Est-2 0.654	Est-4 0.505	30
<i>D. aldrichi</i>	Est-2 0.575	Est-4 0.781	30
<i>D. virilis</i>	Est- α 0.763	Est- β 0.402	31
<i>D. willistoni</i>	ME-2 0.300	ME-1 0.039	23
<i>D. tropicalis</i>	ME-2 0.169	ME-1 0.045	23
<i>D. equinoxialis</i>	ME-2 0.186	ME-1 0.033	23
<i>D. paulistorum</i>	ME-2 0.403	ME-1 0.010	23
<i>Dacus oleae</i>	Est-A 0.762	Est-B 0.583	32
<i>Littorina obtusata</i>	Est-1 0.510	Est-3 0.426	33
<i>Peromyscus polionotus</i>	Est-1 0.343 Est-2 0.313	Est-3 0.144 Est-4 0.047	21
<i>Peromyscus floridanus</i>	Est-1 0.488 Est-2 0.502	Est-4 0.252	34
<i>H. sapiens</i>	ACP ₁ 0.519	ACP ₂ 0 ACP ₃ 0.001	7
<i>H. sapiens</i>	Pep-B 0.002 Pep-C 0.006	Pep-A 0.001 Pep-D 0.014	7

are possible from Table 3, monomers have a higher amount of heterozygosity in 20. Evidently, within a given species or population monomeric enzymes are more polymorphic than multimeric enzymes of similar function.

After the demonstration³⁵⁻³⁶ that populations do indeed contain alleles of same electrophoretic mobility one is not allowed to directly translate electrophoretic variability to actual variability. If polymerisation interferes with the electrophoretic expression of charge changes, then it is possible that amino acid substitutions result in changes in electrophoretic mobility more often in monomers than in multimers. There is evidence, however, that the number of different electrophoretic states that multimeric enzymes can assume is as high as, if not higher than, in monomeric enzymes. Some enzymes with the highest observed numbers of electrophoretic alleles are multimers: esterase B of *Dacus oleae*³² (12 electrophoretic alleles); esterase 5 of *Drosophila pseudoobscura*³⁷ (12); acetaldehyde oxidase and xanthine dehydrogenase of *Drosophila willistoni*²³ (11 and 10, respectively); esterase E of *Colias eurytheme*³⁸ (13); esterase d of *Hemiargus isola*³⁹ (18). In humans the enzyme with the highest observed number of electrophoretic alleles, 18 in all, is the dimeric placental alkaline phosphatase⁴⁰.

Lewontin (ref. 1, p. 71) has stressed the point that small amounts of unconditional overdominance will quickly lead to high degrees of heterozygosity. If heterozygotes for multimeric enzymes are endowed with high fitness and heterozygotes for monomeric enzymes are not, then we would expect multimeric enzymes to possess higher amounts of variation than monomeric enzymes. The opposite is true. Unless one assumes that the variation in multimers is maintained by overdominance, whereas the much higher variation in monomers is neutral or is maintained by some other form of balancing selection, it becomes difficult to adhere to the hypothesis that heteromolecules provide a basis for overdominance of fitness.

The evidence presented here renders the hypothesis of widespread unconditional overdominance less attractive, but it does not exclude the possibility that other forms of heterozygote superiority, such as marginal overdominance⁴¹, are of importance in evolution. Gillespie and Langley²⁶ have shown, however, that if selection coefficients fluctuate greatly as a result of change in the environment, then heterozygote intermediacy can often result in stable polymorphism. Their review of the literature shows that very

rarely, if ever, do heterozygotes assume values above or below the range specified by the two homozygotes. Heterozygote intermediacy is also a concomitant of frequency dependent selection⁴². It seems very possible that, whatever fraction of the genetic variation is selectively maintained, the type of selection involved is one of fluctuations in selection coefficients with no intrinsic heterozygote superiority.

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Genetic transformation in *Drosophila* by microinjection of DNA

THE experiments described here provide new evidence for DNA-induced gene-specific alterations in *Drosophila*. Host embryos homozygous for recessive alleles of several genes were treated with wild-type DNA by embryo microinjection. Several of the

adults which developed from these injected embryos exhibited phenotypic alterations whereas none of the controls treated with host-type DNA showed any altered phenotype. In addition, the alterations were in two cases transmitted to the progeny of the injected flies, so that altered stocks could be established. If this represents genetic transformation and if it can be made to work routinely in eukaryotes, it promises to be equally or more useful than the corresponding process in prokaryotes, since it could be used to bioassay specific parts of the genome which are currently being isolated by various techniques.

Ten years ago Fox and Yoon¹ reported that *Drosophila* embryos soaked in solutions of DNA showed somatic changes in the emerging adults, and subsequently germinal changes², that were specific to the DNA used. In addition to *Drosophila*, the uptake, expression and transmission of exogenous DNA has been reported in both *Neurospora* and mammalian cells *in vitro*, as well as in a number of organisms less suited to further genetic analysis³⁻⁷. The fungal transformation phenomenon was observed after addition of wild-type DNA (or poly(A)-rich RNA) to the growth medium of a fragmented mutant culture⁸⁻¹⁰. The change observed in most studies was an alteration of the organism's dependence on inositol in the culture medium. The frequency of transformation was significantly greater than the observed reversion frequency, either spontaneous or induced, for the mutation. The treatment of cultured mammalian cells with metaphase chromosomes from different species and the subsequent detection of donor enzyme activity in clones derived from the treated cells¹¹⁻¹³ demonstrates further that higher eukaryotes can incorporate and utilise exogenous genetic material.

In *Drosophila*, somatic as well as germ-line alterations have been observed after soaking embryos in solutions of DNA extracted from genetically different animals. The process used to obtain eggs permeable to DNA is not, however, completely satisfactory. It involves inducing animals to lay premature eggs and has been found difficult as well as largely ineffective (less than 2% of the eggs were shown by autoradiography to take up the DNA)¹⁴. In an effort to streamline the transformation experiments (by eliminating the scoring of untreated animals) and to enable some quantification of the treatment, we have adapted an egg microinjection method¹⁵ for this procedure. Although this process limits the number of embryos which can be treated, because of the low survival rate, it allows precision in the delivery of exogenous DNA and serves to increase the chance that a treated egg will be altered genetically.

The genetic outline of our experiment was basically the same as that used by Fox and Yoon². Eggs collected from flies homozygous for the mutations *vermillion*, *v*, and *brown*, *bw*, were used as hosts for DNA extracted from wild-type adults. *Vermillion* and *brown* represent mutations in the ommochrome and pteridine biosynthetic pathways, respectively, and in combination result in the absence of pigment from the adult eyes. Furthermore, *vermillion* is non-autonomous in its expression so that a small patch of non-mutant tissue in the body of the animal can allow the eyes to develop the wild-type phenotype (brown pigmentation) in an otherwise mutant animal. This system, previously exploited by Fox, allows a "magnification" in the detection of first generation alterations, since transformation of tissues other than the eye can be detected by its effect on eye pigmentation. In addition, all hosts were homozygous for *yellow* (a cuticle marker) and most were homozygous for *forked* (a bristle mutation) and *maroon-like* (a mutation which can be detected by lack of staining for aldehyde oxidase in the internal organs). Embryos were injected during the nuclear multiplication stage. During this stage of embryogenesis the nuclei are dividing rapidly (13 divisions at 10-min intervals at 25 °C) and the embryo is a syncytium, that is, no cell membranes separate the nuclei. Injection at this stage should maximise the potential interaction between nuclei and exogenous DNA.

The results of these injections with respect to alterations of *vermillion* expression are summarised in Table 1. A significant number of adults developing from treated embryos had pig-

Table 1 Eye pigmentation in adults developing from embryos injected with DNA

Treatment	Adults emerged	Adults with pigmented eyes	Fertile adults*	Adults with pigmented progeny
<i>v</i> ⁺ DNA	138	8	100	2
<i>v</i> DNA	87	0†	61	0

DNA was extracted from whole adults by a procedure similar to that of Tartof¹⁷. A nuclear preparation was deproteinised using a mixture of chloroform-isoamyl alcohol (24:1) and phenol. The DNA was precipitated, resuspended and treated successively with RNase, Pronase, and phenol. The resulting preparation was dialysed extensively against SSC (0.15 M NaCl, 0.015 M sodium citrate) and then a modified insect Ringer's solution¹⁸. The DNA was then frozen in 100- μ l aliquots at -70 °C. The concentration of DNA injected varied and the results above represent the animals surviving treatment with 44-100 μ g ml⁻¹. Preblastoderm embryos 30-60 min old were injected with 10⁻⁴ μ l DNA through glass needles 10-13 μ m inner diameter. The injected embryos were maintained until hatching at 18-20 °C and the larvae were subsequently raised in yeast paste at 25 °C. The emerging adults were coded, scored, and then allowed to breed for at least 10 d with animals of the host stock. All F₁ progeny were scored for eye colour and 10 pairs of F₁ from each adult were maintained. F₂ and F₃ were similarly scored for deviations from expected eye colour.

*Adults whose progeny were scored at least three generations.

†0.01 < *P* < 0.025.

mented eyes. One of these flies also transmitted the pigmentation to one of his progeny forming a stable transformed line. Another stable line was established from the recovery of pigmented F₂ from an unpigmented injected animal. Genetic analysis of the two transformed lines demonstrated that the alterations were both linked to the X chromosome, which carries the *vermillion* locus. The alteration in one line is dominant and maps near *v* (1-33.2), whereas the other is semi-dominant and maps to the distal end of the chromosome (1-0.01). These results are similar to those of Fox *et al.*¹⁶ and to the preliminary observations made by Limbourg-Bouchon using microinjection (B. Limbourg-Bouchon, personal communication). Neither of these lines showed any instability in the expression of the pigmentation after the first pigmented flies were isolated, nor has there been any reversion in the stocks. This contrasts with the behaviour of stocks produced by other workers where phenotypic instability (interpreted as somatic mosaicism of expression) and reversion are quite common^{2,16}, suggesting that there may be qualitatively different events induced by the DNA treatment. Adults were also examined for alterations in the expression of *y*, *bw*, *f*, and *mal*, but no alteration was observed (*y* and *bw*, 138 cases; *f* and *mal*, 88 cases). The detection of altered expression in these autonomous genes may be significantly less likely than for non-autonomous genes such as *vermillion*. It is also likely, however, that the transformation rate will be characteristic for each gene and that these other genes might simply transform at a lower frequency.

Our results indicate that the phenomenon observed by Fox and Yoon² can be duplicated using egg microinjection. In addition, the frequency of altered expression is significantly greater than that observed by soaking eggs (5% first generation transformants as opposed to 0.8%)². Preliminary results from other laboratories using this technique indicate that an even higher rate may be attainable (B. Limbourg-Bouchon, personal communication; C.P. Liu, and A. S. Fox, personal communication). Microinjection allows precision in delivery, circumvents the uptake phenomenon which may be selective, and allows precise staging of each embryo. Experiments are now under way to transform loci whose genetic fine structure will allow an analysis of the mechanisms underlying this apparent genetic transformation.

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Integrator gene in *Aspergillus nidulans*

ONE of the most intriguing problems in genetics is how the regulation of a particular structural gene can be programmed in such a way that its expression can be elicited in a variety of different contexts. Stated differently, how can the concomitant expression of two or more non-contiguous structural genes capable of independent expression be achieved? Britten and Davidson¹⁻³ have proposed a model for gene regulation which gives considerable insight into this problem. One feature of their model is the existence of positively acting regulatory genes, designated integrator genes, whose role is to achieve precisely this concomitant expression of non-contiguous structural genes. Although the model was developed to account for cell differentiation in higher eukaryotes, a similar, although possibly less elaborate, form of regulatory machinery might be applicable in lower eukaryotes. In the fungus *Aspergillus nidulans*, as in higher eukaryotes, functionally related genes are seldom clustered⁴. Given the considerable metabolic versatility of this organism, instances of inducible enzymes participating in more than one metabolic pathway should not be unduly rare. Evidence for the existence of integrator genes could come from the study of regulatory mutations affecting such enzymes. The model also requires the existence of receptor sites for integrator gene products, a not implausible requirement for *A. nidulans*, where several *cis*-acting regulatory mutations tightly linked to structural genes under their control have been identified (refs 5-8 and unpublished results of C. R. Bailey and H. N. A.). Here I report that the *intA* gene of *A. nidulans*, previously designated *amdR* and shown by Hynes and coworkers⁹⁻¹¹ to be a positive regulatory gene in linkage group II involved in acetamidase synthesis, is an integrator gene of the type described by Britten and Davidson. Moreover, *intA* is possibly especially interesting because one of the compounds whose metabolism it controls is γ -amino-*n*-butyric acid (GABA), whose metabolism has been extensively investigated in a wide variety of organisms because of its role in the regulation of neuronal activity.

Three probable structural genes are under *intA* control: *amdS*, the structural gene for acetamidase in linkage group III⁹⁻¹¹; *gatA* in linkage group VII where recessive mutations

eliminate GABA transaminase which also participates in the catabolism of β -alanine, δ -amino-*n*-valerate, and several other ω -amino acids (H. A. Penfold and H. N. A., unpublished); and *gabA* in linkage group VI where recessive mutations result in loss of a GABA-specific permease (H. A. Penfold and H. N. A., unpublished). *amdA*, another regulatory gene probably specifically controlling *amdS*, is located 5.3 ± 1.8 cM from *gatA*. But there is no interaction in double mutants and no reason to suppose that this linkage has any special significance.

intA mutations can affect expression of one or more of the structural genes. Partially dominant *intA*^c mutations can lead to high, constitutive levels of acetamidase^{9,11} whereas recessive *intA*⁻ mutations halve acetamidase levels⁹. *intA* mutations do not affect the structure of acetamidase¹⁰. *In vivo* effects of *intA* mutations are given in Tables 1 and 2. *intA*^c-2, -300, -302, and -304 enhance to varying extents utilisation of acrylamide and acetamide as nitrogen sources and acetamide as carbon source (Table 1). This enhancement is closely correlated with their abilities to suppress *areA*^r mutations for utilisation of acetamide as nitrogen source (Table 2). *areA*^r mutants lack a positively acting regulatory substance necessary for the synthesis of ammonium-repressible enzymes and consequently are unable to utilise nitrogen sources other than ammonium in glucose-minimal medium^{7,12}. *intA*⁻ mutations somewhat reduce utilisation of acetamide. *amdA*^d mutations enhance acetamide but not acrylamide utilisation and suppress *areA*^r mutations on acetamide. Expression of the *intA*^c and *amdA*^d phenotypes on acrylamide and acetamide media requires a functional allele of the *amdS* but not *gabA* and *gatA* genes. The strict additivity of *intA*⁻-101 and the partially dominant *amdA*^d-301 in strains carrying both mutations (Tables 1 and 2) suggests that, as predicted by Britten and Davidson², *intA* and *amdA* might be regulating *amdS* in a parallel, positive fashion. As *intA*⁻ mutations do not abolish amide inducibility of acetamidase⁹, this form of regulation might involve *amdA*. Selection of *amdA*⁻ alleles and a biochemical study of the effects of *amdA* mutations on acetamidase regulation are intended.

Effects of *intA* mutations on expression of the *gatA* gene are seen in Tables 1 and 3. *intA*⁻ mutations prevent utilisation of GABA, β -alanine, and δ -amino-*n*-valerate as nitrogen sources and GABA as carbon source (Table 1). *intA*^c-2, -300, -302, -304, and -305 slightly enhance δ -amino-*n*-valerate utilisation. Mutants defective in the *ornB* gene in linkage group VIII are blocked before L-ornithine in the biosynthesis of L-arginine and require either ornithine or arginine^{4,13}. GABA and β -alanine but not δ -amino-*n*-valerate can supplement *ornB*⁻ strains (Table 3) but not the arginine/ornithine auxotrophies^{4,13} of *ornA*-4, *ornC*-31, and *ornD*-10 strains or the arginine auxotrophies^{4,13} of *argA*-1, *argB*-2, and *argC*-3 strains. GABA and β -alanine, however, cannot supplement *ornB*⁻ strains also carrying an *intA*⁻ or *gatA*⁻ mutation. *intA*^c-2, -300, -302, -304, and -305 exert, to varying degrees, locus-specific suppression of *ornB*⁻ auxotrophies (Table 3) although not suppressing *ornA*-4, *ornC*-31, *ornD*-10, *argA*-1, *argB*-2, and *argC*-3. This physiological suppression requires that the *gatA* gene but not the *amdS* and *gabA* genes be functional: *intA*^c *gatA*⁻ *ornB*⁻ triple mutants require arginine or ornithine and do not respond to GABA or β -alanine. *ornB*⁻ mutations probably abolish the transaminase which normally converts *N*-acetyl-L-glutamic γ -semialdehyde to *N*⁶-acetyl-L-ornithine. When induced by GABA or β -alanine or rendered constitutive by an *intA*^c mutation, the GABA transaminase can apparently catalyse this conversion.

Expression of the *gabA* permease in *intA* mutants can be followed in Tables 1 and 2. GABA is extremely toxic to *gatA*⁻ mutants. *gabA*⁻ and *intA*⁻ mutations relieve this toxicity by reducing GABA uptake (H. A. Penfold and H. N. A., unpublished) so that *gabA*⁻ *gatA*⁻ and *intA*⁻ *gatA*⁻ double mutants but not *gatA*⁻ single mutants can grow on GABA plus another nitrogen source such as nitrate (Table 1). Given their lack of *gatA* expression, the very growth of *intA*⁻ mutants on GABA plus nitrate also illustrates lack of *gabA* expression. The

Table 1 Growth responses of wild type and mutant strains

Relevant genotype	Acrylamide as N	Acetamide as N	Acetamide as C	GABA as N	GABA + NO ₃ ⁻ as N	GABA as C	β-Alanine as N	δ-Amino-n-valerate as N
Wild type	—	3+	3+	5+	5+	5+	5+	4+
<i>intA</i> ^c -2	4+	5+	5+	5+	5+	5+	5+	5+
<i>intA</i> ^c -300	2+	5+	5+	5+	5+	5+	5+	5+
<i>intA</i> ^c -302	3+	5+	5+	5+	5+	5+	5+	5+
<i>intA</i> ^c -304	2+	5+	5+	5+	5+	5+	5+	5+
<i>intA</i> ^c -305	—	3+	3+	5+	5+	5+	5+	5+
<i>amdA</i> ^d -301, -303	—	5+	5+	5+	5+	5+	5+	4+
<i>intA</i> ^c -2 <i>amdA</i> ^d -301	4+	5+	5+	5+	5+	5+	5+	5+
<i>intA</i> ^c -304 <i>amdA</i> ^d -301	3+	5+	5+	5+	5+	5+	5+	5+
<i>intA</i> ⁻ -44, -100, -101, -105, -106, -107	—	2+	+	—	5+	—	—	±
<i>intA</i> ⁻ -500	—	2+	+	3+	5+	3+	—	4+
<i>amdA</i> ^d -301 <i>intA</i> ⁻ -101	—	3+	3+	—	5+	—	—	±
<i>amdS</i> -17	—	—	—	5+	5+	5+	5+	4+
<i>gabA</i> -2, -6	—	3+	3+	2+	5+	±	5+	4+
<i>gatA</i> -1, -2	+	4+	4+	—	—	—	—	—
<i>gabA</i> -2 <i>gatA</i> -1, -2	+	4+	4+	—	3+	—	—	—
<i>intA</i> ⁻ -101 <i>gabA</i> -2	—	2+	+	—	5+	—	—	±
<i>intA</i> ⁻ -101 <i>gatA</i> -1, -2	—	2+	+	—	3+	—	—	—
<i>intA</i> ⁻ -500 <i>gabA</i> -2	—	2+	+	2+	5+	±	—	4+
<i>intA</i> ⁻ -500 <i>gatA</i> -1	—	2+	+	—	2+	—	—	—
<i>intA</i> ^c -2 <i>amdS</i> -17	—	—	—	5+	5+	5+	5+	5+
<i>intA</i> ^c -2 <i>gabA</i> -2	4+	5+	5+	2+	5+	±	5+	5+
<i>intA</i> ^c -2 <i>gatA</i> -1, -2	4+	5+	5+	—	—	—	—	—
<i>amdA</i> ^d -301 <i>amdS</i> -17	—	—	—	5+	5+	5+	5+	4+
<i>amdA</i> ^d -301 <i>gatA</i> -1	+	5+	5+	—	—	—	—	—

—, No growth; ±, very slight growth; + up to 5+, increasing levels of growth. Growth scores on different media are not necessarily equivalent, but 5+ represents the maximum growth observed on any medium.

Growth tests were carried out at 37 °C on appropriately supplemented minimal medium¹⁵ as described previously¹⁶. At least several different strains of each relevant genotype were tested. Nitrogen sources were added to 1% D-glucose-containing media at 10 mM (acrylamide, acetamide, sodium nitrate) or 5 mM (β-alanine, GABA, δ-amino-n-valerate). Carbon sources were added to a final concentration of 50 mM to glucose-free media. Growth scores on different media are not necessarily equivalent. All strains listed above utilise L-glutamate and L-aspartate normally as nitrogen or carbon sources.

intA^c-2 (formerly *amdR*^c-2), *intA*⁻-44 (formerly *amdR*⁻-44), and *amdS*-17 were provided by Dr M. J. Hynes, who has described their selection⁹. *intA*^c-300, *amdA*^d-301, and *intA*^c-302 (formerly designated *amdA*^d-300, -301, and -302) were obtained as suppressors of *areA*^r-1 on acetamide as described previously¹². *intA*^c-304, *amdA*^d-303, and *intA*^c-305 were obtained after N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagenesis¹⁷ of a strain of genotype *biA*-1 *areA*^r-2 *fwA*-1 (biotin-requiring, unable to utilise nitrogen sources other than ammonium, fawn conidial colour) as suppressing *areA*^r-2 on acetamide, acetamide, and GABA as nitrogen sources, respectively. *intA*⁻-100 and -101 were obtained as spontaneous mutations in strains of genotype *pabaA*-1 *intA*^c-2 *mauA*-2 (*p*-aminobenzoate-requiring, *intA*^c-2 phenotype as described herein, lacking monoamine oxidase) and *puA*-2 *intA*^c-300 (putrescine-requiring, *intA*^c-300 phenotype as described herein), respectively, resulting in resistance to 4 mg ml⁻¹ 2-fluoroacetamide (Fluka, Buchs, Switzerland) on medium containing 72 mM acetate (pH 6.5) as sole carbon source and 5 mM urea as sole nitrogen source. *gabA*-2 and -6 were selected as unable to utilise 5 mM GABA as nitrogen source after NTG mutagenesis of a strain of genotype *biA*-1 *areA*^r-2 *fwA*-1 using the putrescine starvation selection technique¹⁸. *intA*⁻-105, -106, -107, and -500 and *gatA*-1 were selected after ultraviolet mutagenesis of a strain of genotype *pabaA*-1, using replica plating¹⁹, as able to utilise 5 mM L-proline but not 5 mM β-alanine as nitrogen source. *gatA*-2 was selected by Mr K. N. Rand. It was obtained after ultraviolet mutagenesis of a strain of genotype *pabaA*-1 *areA*^r-120 as allowing utilisation of 10 mM acrylamide as nitrogen source in the presence of 1% (v/v) glycerol as sole carbon source. This weak suppression is presumably associated with the slight enhancement of acetamide and acrylamide utilisation by *gatA*⁻ mutations (which will nevertheless not suppress the more stringent *areA*^r alleles on acetamide or acrylamide) as seen above. A likely explanation is that *gatA*⁻ mutations enhance the accumulation of an endogenous inducer such as β-alanine which can activate the *intA* product, as no such enhancement occurs in strains also carrying an *intA*⁻ mutation.

Allelism has been demonstrated both by tight linkage (generally less than 0.5 cM) and, for recessive mutations resulting in loss of function, by a lack of complementation in diploids. The leakiest *intA*⁻ allele, *intA*⁻-500, does not complement with *intA*⁻-44, -100, -101, -105, -106, or -107. The *intA*⁻ mutations are recessive to *intA*⁺ on the media listed above whilst *intA*^c-2 is partially dominant to both *intA*⁺ and *intA*⁻-101.

ability of the five *intA*^c mutations to suppress *areA*^r for utilisation of GABA as nitrogen source (Table 2) probably primarily reflects enhanced levels of the *gabA* permease because suppression of *areA*^r on GABA can also be achieved by a *cis*-dominant regulatory mutation tightly linked to *gabA* (unpublished results C. R. Bailey and H. N. A.). Suppression of *areA*^r on GABA requires functional alleles of both *gabA* and *gatA* but not *amdS*. The selection of mutations able to suppress *areA*^r mutations on two or more nitrogen sources metabolised by different pathways is probably a general method for obtaining derepressed alleles of integrator genes, provided synthesis of the necessary enzymes and permeases does not have an absolute requirement for a functional *areA* product (K. N. Rand and H. N. A., unpublished).

The probable route of GABA catabolism is shown in Fig. 1. Although *intA* controls both GABA uptake and transamination, it apparently does not regulate succinic semialdehyde dehydrogenase because *intA*⁻ strains utilise γ-hydroxy-n-butyrate as a carbon source. *ssuA*-1 strains can utilise neither GABA nor γ-hydroxy-n-butyrate as carbon sources (C. R. Bailey and H. N. A., unpublished).

As well as controlling three structural genes, the *intA* product must respond to at least two effectors, GABA and β-alanine or their derivatives, as shown by their ability to supplement *ornB*⁻ auxotrophies. If the *intA* product be directly involved in regulating *amdS*, *gatA*, and *gabA*, mutations affecting the specificity of the *intA* product might fall into one or both of two categories: (1) those having a differential effect on the expression of the three structural genes; and (2) those affecting response to one of the effectors more than the other. *intA*⁻-500 is probably of the latter type since it affects responses to β-alanine much more drastically than responses to GABA or δ-amino-n-valerate (Tables 1 and 3). The lack of additivity of *intA*⁻-500 and *gabA*-2 in double mutants and the relative resistance of *intA*⁻-500 *gatA*-1 double mutants to GABA supports this conclusion. The fully *intA*⁻ phenotype of *intA*⁻-500 on acetamide (Table 1) plus its resistance to acetamidase-catalysed, lethal hydrolysis of 2-fluoroacetamide⁹ might indicate that *intA*⁻-500 also falls into the first specificity category, stringently affecting *amdS*. It is more likely, however, that the mutant responses of *intA*⁻ strains to acetamide and its analogues are dependent on a small amount of endogenous effector

Table 2 Suppression of *areA*⁺ mutations

Relevant genotype	δ -Amino- <i>n</i> -valerate or β -alanine or NO_3^-	Nitrogen source		
		Acetamide	GABA	NH_4^+
Wild type (<i>areA</i> ⁺)	5+	5+	5+	5+
<i>areA</i> ⁺ -1, -2, -19	—	—	—	5+
<i>intA</i> ^c -2 <i>areA</i> ⁺ -1, -2, -19	—	4+	3+	5+
<i>intA</i> ^c -300 <i>areA</i> ⁺ -1	—	3+	2+	5+
<i>intA</i> ^c -302 <i>areA</i> ⁺ -1	—	3+	+	5+
<i>intA</i> ^c -304 <i>areA</i> ⁺ -2	—	3+	±	5+
<i>intA</i> ^c -305 <i>areA</i> ⁺ -2	—	—	3+	5+
<i>amdA</i> ^d -301 <i>areA</i> ⁺ -1, -2, -19	—	3+	—	5+
<i>amdA</i> ^d -303 <i>areA</i> ⁺ -2	—	3+	—	5+
<i>intA</i> ^c -304 <i>amdA</i> ^d -301 <i>areA</i> ⁺ -2	—	4+	±	5+
<i>intA</i> ^c -2 <i>amdS</i> -17 <i>areA</i> ⁺ -1	—	—	3+	5+
<i>intA</i> ^c -2 <i>gabA</i> -2 <i>areA</i> ⁺ -2	—	4+	—	5+
<i>intA</i> ^c -2 <i>gatA</i> -2 <i>areA</i> ⁺ -2	—	4+	—	5+
<i>amdA</i> ^d -301 <i>amdS</i> -17 <i>areA</i> ⁺ -1	—	—	—	5+
<i>amdA</i> ^d -301 <i>intA</i> ⁺ -101 <i>areA</i> ⁺ -2	—	+	—	5+
<i>intA</i> ⁺ -101 <i>areA</i> ⁺ -1, -2	—	—	—	5+
<i>amdS</i> -17 <i>areA</i> ⁺ -1	—	—	—	5+
<i>gabA</i> -2 <i>areA</i> ⁺ -2	—	—	—	5+
<i>gatA</i> -2 <i>areA</i> ⁺ -1, -2	—	—	—	5+

See legend to Table 1 for most details. Ammonium was added at 10 mM as the (+)-tartrate. *areA*⁺-1, -2, and -19 (formerly *amdT*-19) have been described previously¹² and lead to inability to utilise nitrogen sources other than ammonium. Growth scores on different media are not necessarily equivalent. When tested in diploids homozygous for *areA*⁺-2, *intA*^c-2 and *amdA*^d-301 are partially dominant. None of the *intA*^c or *amdA*^d mutations suppress *areA*⁺ mutations on L-glutamate or L-aspartate as nitrogen sources.

interacting with the *intA* product in *intA*⁺ strains. The behaviour of *intA*⁺-500 might therefore suggest that β -alanine, an essential metabolite for synthesis of coenzyme A, is that effector. Its concentration must be sufficiently low for *ornB*⁻ mutations to result in complete auxotrophy. Low levels of β -alanine and GABA have been detected in *Neurospora crassa*¹⁴.

intA^c-305 and -304 are probably specificity mutations of the first category. *intA*^c-305 strongly affects *gabA* expression

but has little effect on expression of *amdS* and *gatA*. The opposite is true of *intA*^c-304. This indicates that the *gabA* receptor site for the *intA* product differs from those of the *amdS* and *gatA* genes. A diversity of receptor sites for the *areA* product has already been postulated⁷. The existence of such opposite phenotypes among *intA* alleles is only compatible with direct regulatory involvement of the *intA* product. Indirect involvement could only lead to a hierarchy of structural gene

Table 3 Supplementation and suppression of *ornB*⁻ mutations

Relevant genotype	L-Arginine or L-ornithine	Supplement		
		β -Alanine	GABA	None
Wild type (<i>ornB</i> ⁺)	5+	5+	5+	5+
<i>ornB</i> ⁻ -7, -8, -9, -20, -300	5+	4+	3+	—
<i>intA</i> ^c -2 <i>ornB</i> ⁻ -7, -8, -9, -20, -300	5+	5+	5+	5+
<i>intA</i> ^c -300 <i>ornB</i> ⁻ -7	5+	5+	5+	2+
<i>intA</i> ^c -302 <i>ornB</i> ⁻ -7	5+	5+	5+	4+
<i>intA</i> ^c -304 <i>ornB</i> ⁻ -7	5+	5+	5+	3+
<i>intA</i> ^c -305 <i>ornB</i> ⁻ -7	5+	5+	5+	±
<i>amdA</i> ^d -301 <i>ornB</i> ⁻ -7	5+	4+	3+	—
<i>intA</i> ^c -2 <i>amdS</i> -17 <i>ornB</i> ⁻ -7	5+	5+	5+	4+
<i>intA</i> ^c -2 <i>gabA</i> -2 <i>ornB</i> ⁻ -7	5+	5+	5+	4+
<i>intA</i> ^c -2 <i>gatA</i> -1, -2 <i>ornB</i> ⁻ -7	5+	—	—	—
<i>amdS</i> -17 <i>ornB</i> ⁻ -7	5+	4+	3+	—
<i>gabA</i> -2 <i>ornB</i> ⁻ -7	5+	4+	3+	—
<i>gatA</i> -1, -2 <i>ornB</i> ⁻ -7	5+	—	—	—
<i>intA</i> ⁺ -101 <i>ornB</i> ⁻ -7	5+	—	—	—
<i>intA</i> ⁺ -500 <i>ornB</i> ⁻ -7	5+	—	+	—

See legends to Tables 1 and 2 for most details. Supplementation by L-arginine and L-ornithine (as the monohydrochlorides) was tested at 5 mM with or without another nitrogen source. Unsupplemented medium contained 10 mM ammonium as nitrogen source. β -alanine and GABA were added with or without uric acid (100 $\mu\text{g ml}^{-1}$) although they definitely supplement more efficiently in the absence of another nitrogen source. GABA supplementation of *ornB*⁻ strains is considerably improved by using strains also carrying a mutation relieving ammonium repression such as *meaA*-8^{16,20} or *areA*^d-102 (formerly *amdT*-102)¹² or by using glucose-free medium with 50 mM GABA as sole carbon source. Presumably, self nitrogen metabolite repression¹² limits GABA supplementation of *ornB*⁻ single mutants growing on GABA as sole nitrogen source. Neither L-glutamate nor L-aspartate supplements *ornB*⁻ auxotrophies.

The arginine/ornithine auxotrophies *ornB*⁻-7, -8, -9, and -20 are markers in standard use⁴. *ornB*⁻-300 was selected after ultraviolet mutagenesis of a strain of genotype *yA*-2 *alX*-4 *pantoB*-100 (yellow conidial colour, lacking allantoinase, requiring pantothenic acid), using replica plating, as able to grow on a medium containing 5 mM GABA but not 5 mM L-proline as nitrogen source. *ornB*⁻-300 is temperature sensitive, resulting in auxotrophy at 37 °C but not at 25 °C. It is tightly linked to *ornB*⁻-7, and they do not complement in diploids.

When diploids homozygous for *ornB*⁻-7 are tested on the above media, *intA*^c-2 is partially dominant whereas *intA*⁺-101 is recessive.

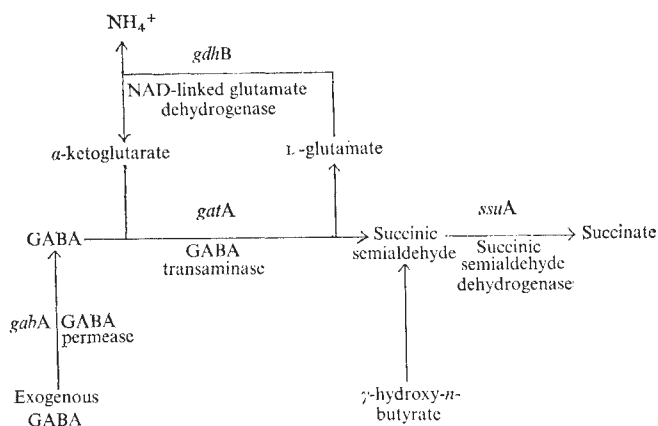


Fig. 1 The pathway of GABA catabolism in *A. nidulans*, shown above, is probably very similar to that in *Escherichia coli*²¹. As expected^{22–25} for compounds whose nitrogen is metabolised by way of L-glutamate before ammonium, GABA, β-alanine, and δ-amino-n-valerate supplement *gdhA*− mutants lacking the anabolic NADP-linked glutamate dehydrogenase, but are not utilised by *gdhB*− mutants lacking the catabolic NAD-linked glutamate dehydrogenase. GABA can also supplement *pycA*− mutants²⁶ lacking pyruvate carboxylase and consequently requiring a source of a four-carbon dicarboxylic acid. The utilisation of GABA is subject to both nitrogen metabolite¹² and carbon catabolite²⁷ repression (H. A. Penfold, C. R. Bailey, and H. N. A.).

expression. This type of evidence has also shown direct regulatory involvement of the *areA* gene product^{7,12}.

A full biochemical and genetical analysis of α-amino acid catabolism and effects on acetamidase synthesis is in progress (H. A. Penfold, C. R. Bailey, and H. N. A., unpublished). We are particularly interested in seeing whether, as implied by results presented here, the binding of different effectors to the *intA* product alters the relative efficiencies of expression of the three structural genes.

In conclusion, *intA* is a positive regulatory gene whose product directly affects the expression of three unlinked structural genes which can participate in different catabolic pathways. The structural genes code for an acetamidase, a GABA transaminase, and a GABA permease. At least the acetamidase is subject to an alternative, parallel mechanism of induction, involving the specific regulatory gene *amdA*, in addition to the induction mediated by the *intA* product. Therefore *intA* can be described as an integrator gene. The ability of (mostly point) mutations in *intA* to affect expression of any one or two or all three of the structural genes controlled by *intA* argues that *intA* is a single gene, not a battery of coordinately controlled contiguous genes. Of the alternative regulatory circuits offered by Britten and Davidson¹, *intA* therefore fits into the redundancy of receptor sites model rather than the redundancy of integrator genes model.

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Note added in proof: Confirming a crucial prediction, β-alanine and GABA induce acetamidase in wild-type but not in *intA*−101 strains. β-alanine induces to a considerably higher level than acetamide, but simultaneous addition of β-alanine and acetamide results in only an intermediate, not an additive, level of induction. This probably indicates competition between the *intA* product (activated by β-alanine) and the *amdA* product (activated by acetamide) in the induction of acetamidase. Such competition could result from a partial or complete overlap of receptor sites.

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X-ray study of the rubidium salt of ADP

ENZYMATIC reactions involving ATP and ADP require various metal ions as cofactors for catalytic activation. To understand the molecular mechanisms involved in these reactions it is important to know both the detailed geometry of these molecules and the way in which they bind to metal ions. Although numerous physical and chemical studies have been undertaken, particularly of divalent metal complexes in solution, the conformational and electronic aspects of these complexes are not fully understood. In the solid state, crystallographic studies have led to deductions on the conformations of ATP and ADP when bound to enzymes (refs 1–3 and H. C. Watson, personal communication) but the only crystal structures so far determined to atomic resolution are of the disodium salt of ATP⁴ and the monorubidium salt of ADP⁵. The latter structure has so far only been published in part although some of its molecular features were reviewed recently⁶.

The work reported here is on a different hydrate of monorubidium ADP containing one water molecule per nucleotide instead of three as in the earlier study. The Rb-ATP and ADP complexes are of biochemical importance as many enzyme systems associated with phosphate transfer require, in addition to divalent metal ions, univalent cations for activation (for example, K⁺, Rb⁺, NH₄⁺)⁷. As part of our study of phosphate coenzymes we have attempted the preparation of single crystals of other mono- and divalent salts of ADP but so far without much success.

The monorubidium salt of ADP was crystallised as needles from water–alcohol solutions containing ADP free acid and RbBr (1:1). The solution was kept at 5 °C and pH 5.5 using Tris buffer. The space group (P2₁2₁2) and cell dimensions (*a* = 28.516(3) Å, *b* = 10.594(3) Å, *c* = 6.315(2) Å) agreed with those reported for the trihydrated rubidium salt by Muller and Deluke⁶. The measured density (*D_m* = 1.86 g cm^{−3}, *D_x* = 1.84 g cm^{−3}, *Z* = 4) indicated, however, the presence of only one water molecule per ADP molecule and this was later confirmed by the structure analysis.

A total of 1,440 unique reflections were collected on a

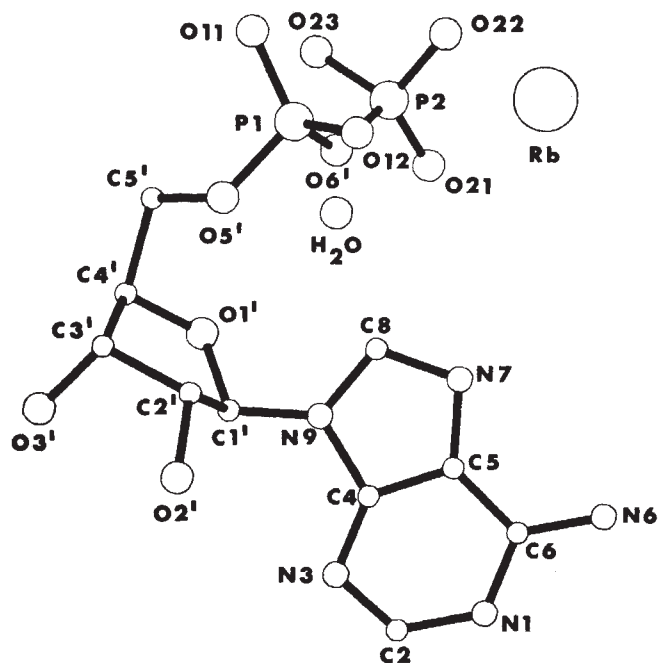


Fig. 1 View of the ADP molecule perpendicular to the base.

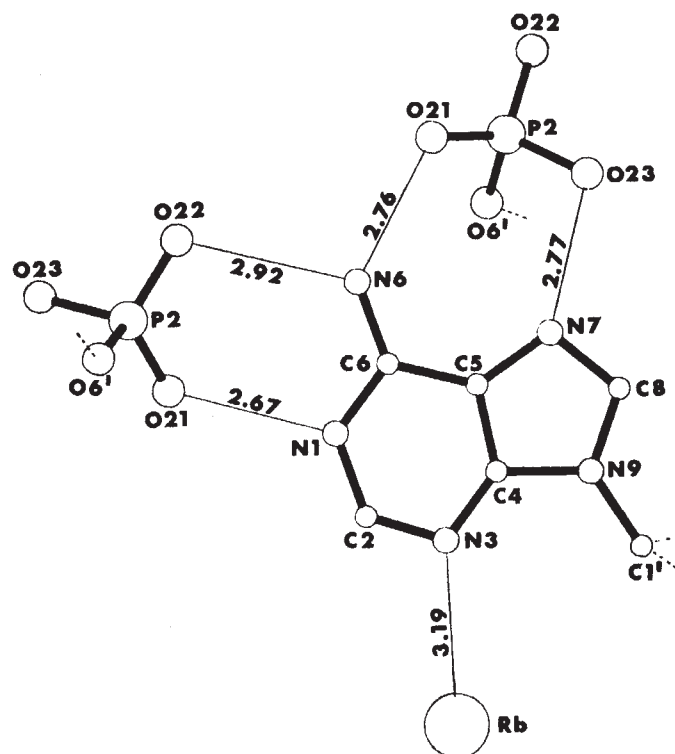


Fig. 2 Interactions involving the base atoms.

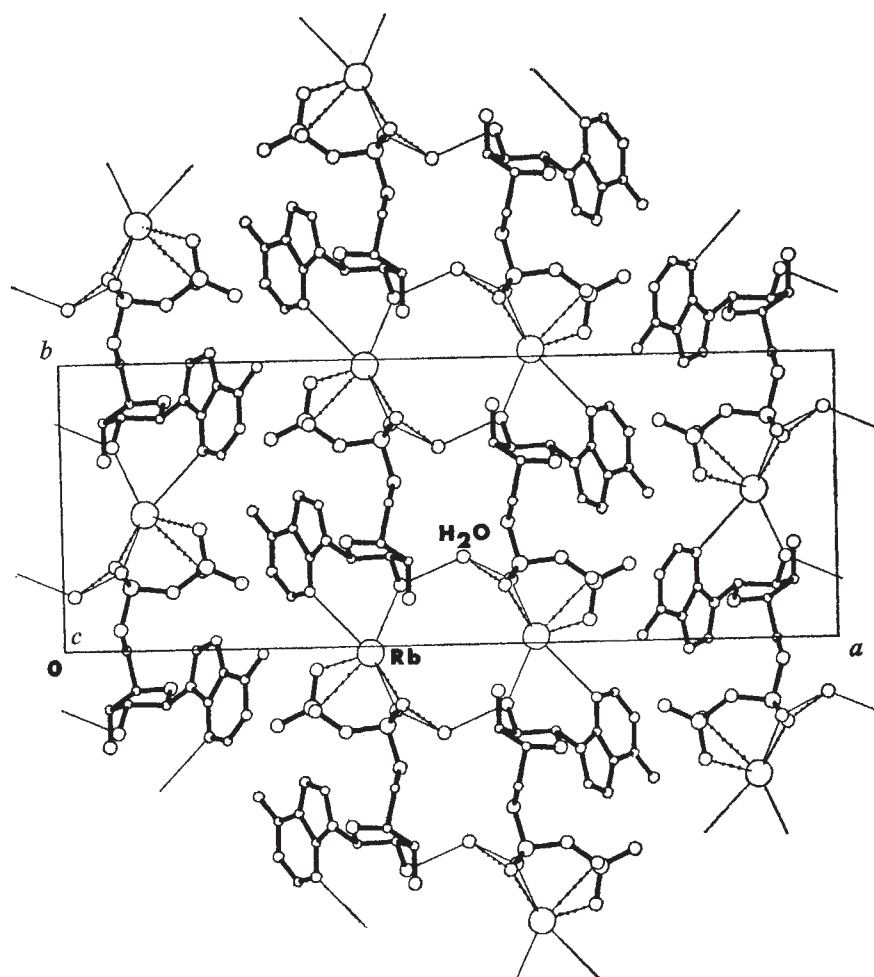


Fig. 3 Crystal packing viewed down the *c* axis showing the Rb^+ coordination and the hydrogen bonding with water molecules. (The dotted lines refer to contacts with molecules related by *c* translation.)

Syntax P₂ diffractometer of which 1,200 were significant on the basis of $F > 3\sigma(F)$. The data were of moderate accuracy due to the poor quality of the crystal specimen.

The structure was solved by the application of MULTAN⁸ and a difference Fourier synthesis and refined anisotropically in a full-matrix least squares program to $R = 11.9\%$.

A view of the molecule, perpendicular to the base, is shown in Fig. 1. The orientation of the base about the glycosidic C1'–N9 linkage is *anti*, the only conformation so far observed in the crystal structures of nucleotides. The dihedral angles O1'–C1'–N9–C8 and C2'–C1'–N9–C8 are 40.3° and –75.8°.

In the ribose ring, C2' is displaced by 0.54 Å from the mean plane through C3', C4', O1', C1' corresponding to a typical C2'-endo puckering. The conformation about the C4'–C5' bond is *gauche gauche* with torsion angles O5'–C5'–C4'–C3' = 57.0° and O5'–C5'–C4'–O1' = –63.3°. The conformation about C5'–O5' is *trans* (P1–O5'–C5'–C4' = 147.1°). A similar conformation has also been proposed as the predominant structure of ADP in solution, from a Fourier transform nuclear magnetic resonance (NMR) study⁹. The pyrophosphate moiety has a staggered conformation. A striking feature of the structure is the observed difference of 0.10 Å between the lengths of the two bridging bonds in the high energy linkage, which seems to be significant at the level of accuracy attained in the present analysis. The values for P1–O6' and P2–O6' are 1.54 and 1.64 Å respectively with an estimated s.d. of 0.02 Å. The angle P1–O6'–P2 is 135° (e.s.d. 1°). Corresponding values in other nucleoside diphosphates recently determined by us are 1.60 and 1.64 Å and 133° in cytidine diphosphocholine and 1.58 and 1.62 Å and 128° in cytidine diphosphoric acid¹⁰.

In the crystal structure, the ADP molecule is folded but there is no Rb⁺ bridge between the phosphate and adenine groups of the same molecule. Nor is there any evidence of other types of intramolecular interactions such as hydrogen bonding or metal binding between the pyrophosphate chain and the base or sugar moiety. In this respect, the ADP structure resembles the conformations found in the crystal structures of the dipotassium salt of uridine-5'-diphosphate (M.A.V., M. L. Post and O.K., unpublished) and the monosodium salt of cytidine diphosphocholine¹⁰. These coenzyme molecules, which are in very different crystalline environments, are also folded without any intramolecular metal ligation. They seem to take up the folded conformation preferentially and it is likely that any binding involving metal ions merely helps to stabilise the folded structure. A recent NMR study of ATP bound to lanthanide cations in aqueous solution¹¹ has been interpreted in terms of a folded conformation, with the lanthanide ion bound only to the β and γ phosphates but with no direct interaction with the purine ring.

It is interesting to note that in contrast to the folded conformation reported here, difference electron density maps suggest that ADP is in the extended form when bound to either lactate dehydrogenase¹ or phosphoglycerate kinase (refs 2, 3 and H. C. Watson, personal communication).

The extended crystal structure shows no self association between the bases through either hydrogen bonding, or base stacking. Instead there are two pairs of hydrogen bonds linking the adenine base to phosphate group of two neighbouring molecules (Fig. 2). One of these pairs has the Watson–Crick geometry, while the other involves the amino group and the imidazole nitrogen N7. These interactions and the binding of Rb⁺ to N3 may be relevant to the recognition of the base by polar side groups of amino acids and metal ions in biological systems (to be published). The role of similar interaction in the sequence specific recognition of nucleic acids by proteins has recently been discussed by Seeman *et al.*¹².

Figure 3 shows the role of the Rb⁺ ions and the water molecules. Each Rb⁺ ion is bound to two ADP molecules through the negatively charged α and β phosphate oxygen atoms. The four Rb ··· O distances range from 2.85 to 3.33 Å. The sixfold coordination around Rb⁺ is completed by N3 of the adenyl base of a third molecule at 3.19 Å and the ribose

hydroxyl atom of the same molecule at 2.91 Å.

The water molecules lie in sheets perpendicular to the *a* axis interlinking layers of Rb–nucleotide complexes through hydrogen bonding.

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Corrigendum

In the article “Two short lived X-ray transients at high galactic latitude” by B. A. Cooke (*Nature*, **261**, 564; 1976) the scale and legend to Fig. 2a are in error. The scale should read 13 h not 3 h and the source designation in the legend should be A1353–40 not A0353–40.

Errata

In the article “Extinction-free measurements in crystallography” by A. McL. Mathieson (*Nature*, **261**, 306; 1976), a sentence was omitted in the editing process. On line 12 of paragraph 1 mention of refs 5–7 should be deleted, and line 13 *et seq.* should read:

‘... is applicable. The possibility exists of extrapolating to this condition, as a practical procedure, in relation to asymmetric reflection^{5–7}. Starting with ...’

In paragraph 4 line 7 of the right-hand column of page 307 ‘ref. 8’ should be ‘ref. 9’, and in the caption to Fig. 2 the reference on the first line should be ‘5’.

In the article “Phosphorylation of yeast RNA polymerases” by G. Bell, P. Valenzuela and W. J. Rutter (*Nature*, **261**, 429; 1976) symbols were omitted from Fig. 1a which make it difficult to understand. The correct symbols are as follows. ³²P c.p.m. (○); RNA polymerase activity (●); Protein concentration (—).

In the article “Simple mathematical models with very complicated dynamics” by Robert May (*Nature*, **261**, 459–467; 1976) Figs 2 and 3 were transposed. The captions were in the correct position.

reviews

Introducing nuclear power

Lord Hinton of Bankside

Nuclear Power. (A Pelican Original.) By Walter C. Patterson. Pp. 304. (Penguin: Harmondsworth, Middlesex, March 1976.) 80p.

WALTER PATTERSON'S book is written in two parts. The first, which he calls "The World of Nuclear Fission" is excellent; I have never read a better simple explanation of the theory and practice of nuclear reactors and their ancillary plants. I am less enthusiastic about the second part of the book, most of which deals with the hazards of nuclear power. It seems to me that, in his anxiety to make a case which is real, Patterson may be guilty of lack of balance. The impression which is given by the italic printing of some paragraphs is that they have been abstracted from official reports; my limited inside knowledge contradicts this impression. For instance, I have never heard the filters on the Windscale piles referred to as "Cockcroft's folly". The stacks had not been built (as stated in italic script) when information from America made it clear that filters were a necessary precaution.

These statements are, in themselves, of minor importance but they throw some doubt on italic accounts of other incidents. The italic paragraph about "the dramatic gesture of pouring away milk" after the Windscale incident seems to be romanticised; I had left the UK Atomic Energy Authority at the time of the incident and knew nothing of any discussions that took place but (although the milk could safely have been used in milk chocolate or as dried milk) it seems to me that it would have been a foolish firm which bought the milk and laid itself open to the unfounded accusation of selling a radioactive product. The italic paragraphs about the Enrico Fermi fast reactor and mention of the "China syndrome" are hardly a fair argument against the fast reactors which are being designed today and which use a ceramic and not a metallic fuel.

The book does not emphasise the world's real need for nuclear power. The rate of usage of exhaustable natural resources does not increase exponentially to a sharp peak and suddenly fall away to nothing; it conforms to a wave-shaped curve so that at some point in time growth becomes steadily slower until a peak is reached; and,

thereafter, growth becomes negative (that is, the rate of usage diminishes).

There is good evidence to suggest that global resources of fossil fuels are such that we shall pass over the peak of such a curve at about the end of this century. It is highly improbable that renewable energy resources (wave power, solar energy and wind power) can make up the deficit; without nuclear power it is almost certain that standards of living will fall.

Nuclear power does give rise to some hazards but nothing in this world is absolutely safe. Nuclear power plants can be made as safe or safer than very many other industrial plants provided that engineers are not over-ambitious in extrapolating either the design parameters or the size of the construction programs. With these reservations I

would sooner accept the hazards of nuclear power than the risk of an energy famine.

The first part of Walter Patterson's book is an outstandingly good introduction to nuclear power for the intelligent layman. As a reminder of their responsibilities, Part 2 should be compulsory reading for those men who have responsibilities in the nuclear power industry; but its lack of balance may give a wrong impression to the lay-reader. □

Lord Hinton of Bankside is Deputy Chairman of the Electricity Supply Research Council and was formerly Managing Director (Industrial Group) of the UKAEA and Chairman of the UK Central Electricity Generating Board.

Macrophage house of fashion

Immunobiology of the Macrophage. Edited by David S. Nelson. Pp. xviii + 633. (Academic: New York and London, April 1976.) \$39; £21.45.

As the hemlines of Western ladies rise and fall and split and waver with each new season's modish rhythm so do the fads and fancies of immunologically orientated biologists. Thus lymphocytes were 'discovered' in the sixties and are probably presently at their apogee of man-hour involvement. The macrophage was discovered at the turn of the century but, as Cohn puts it in the Introduction to this fascinating book, the doldrums soon descended on all but a few dedicated phagists. The book heralds the new golden age of the macrophage (compare: Golden Age of Thymology; Miller, *Lancet*, 2, 1299; 1967).

Some of the 24 chapters, each written by a different expert in the field, deal with macrophage involvement in various *in vitro* cell preparations; others with the heterogeneity of macrophages as determined by their site of origin and the complexities of their cell surfaces. The responses of macrophages to chemotactic factors and their capacities to produce and liberate factors which affect other cellular components of the immunological or-

chestra are described. Many are the schemes of cell interaction. The papers by Volkman on monocytes, by Evans and Alexander on macrophage cytotoxicity, by Holtermann *et al.* on immunotherapy of skin tumours and that by Territo and Cline on macrophage disorders in man are all at the end of the book and all well worth reading.

The changes of tempo and topic make me long for the earlier one-man macrophage book of Nelson but he, although remaining an editor, has abandoned the role of a colossus in the field capable of comprehensive review. In spite of this dereliction of function it is Nelson's summarising chapter 9 and final short chapter which are most useful to the outsider. As he says "In the evolution of our complex mammalian system they (macrophages) seem almost ubiquitous, emerging from obscurity to make some lysozymes contribute to the complement system, help a T cell, present a stimulus to a B cell, or quell an unruly mutant."

Those with time and deep pockets (do all books cost £20 these days?) should buy this book. It is well produced, well edited and most informative. But perhaps more important its mastery will put you in on the ground floor of the current macrophage house of fashion.

A. J. S. Davies

Laser physics

Laser Spectroscopy: Proceedings of the Second International Conference, Megève, June 1975. (Lecture Notes in Physics, Vol. 43). Edited by S. Haroche, J. C. Peibay-Peyroula, T. W. Hänsch and S. E. Harris. Pp. x+468. (Springer: Berlin and New York, 1975.) DM 45; \$18.50.

THE development of tunable laser systems has radically altered the nature of atomic and molecular physics. Used in conventional spectroscopy, the high intensity and monochromaticity of the tunable laser makes it an ideal probe of purely atomic properties such as excited state lifetimes, fine and hyperfine structure splittings and isotope shifts: the list of worthy results is endless. When the laser is used in a dynamic sense, new and exciting non-linear, intensity-dependent features emerge. Finally the extremely short duration of pulses generated by pulsed mode-locked lasers enables the photochemist to study ultrafast relaxation and transport mechanisms. This volume, demonstrates how laser spectroscopy has arrived at a semblance of maturity after a period of very rapid growth. The topics discussed here extend from the quantum electrodynamics of laser resonance fluorescence to laser iso-

tope separation processes of economic importance. Invited papers presented at the conference are here printed directly from the authors' typescripts.

For me, some especially interesting papers were those of Cagnac on high resolution, two photon spectroscopy, Mollenauer on colour centre lasers, and Cohen-Tannoudji's review of the currently fashionable dynamic Stark effect in resonance fluorescence. Workers in laser physics or atomic physics will find much in this volume to entertain and to stimulate. One fascinating feature is the large number of papers, some with carefully spelled-out background histories and dates, devoted to laser isotope separation. On this the editors make the curious comment: "Unfortunately, the free flow of information was here still severely hampered by secrecy and classification, and the wisdom of including such a 'political' topic in the program has since been seriously questioned".

No doubt some of the new results presented at the conference for the first time will either appear or already have appeared elsewhere in the literature. But as a review of the status of the field, this volume serves a very useful purpose and can be recommended to workers in atomic and laser physics.

Peter Knight

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Sound basis for plant virology course

Plant Virology: The Principles. By Adrian Gibbs and Bryan Harrison. Pp. x+292. (Arnold: London, March 1976.) £16.

IN the Preface to this excellent book the authors describe its gestation period as 'lengthy', which is scarcely more informative than a piece of string; but as they state that they aimed to provide a text at the university teaching level, a need not filled by books which have since appeared, I suppose that this one grew from notes prepared for lectures given to students of the Imperial College, University of London, by Gibbs and Harrison some 10 years ago while they were both at Rothamsted. This speculation is relevant to an assessment, since a gestation period of several years would account for some characteristics which might otherwise puzzle or even irritate a reader. I am not suggesting that the book is out of date; far from it; I am referring to differences between chapters in style and depth. Problems of selection would have grown erratically as reference

lists lengthened because research papers proliferated on fashionable topics; as the cost of publication rose at an accelerating rate, hard choices would have to be made.

The layout of the book provides a sound basis for a lecture course for undergraduates. After the history and scope of plant virology, some groups of viruses and the cryptogram system are described, followed by accounts of virus effects on plants, and methods of experimental transmission. The next chapter deals with the composition and structure of plant viruses in considerable depth and seems over-elaborate compared with others. Purification and properties are well covered in chapter 6, although the use of liquid nitrogen and of polyethylene glycol in extraction seem strange omissions. Assay methods are clearly described in three chapters separately considering infectivity, serology and physicochemical methods. After chapters on effects of inactivation and behaviour of viruses in plants, one on variation, strains and classification seems misplaced between them and those on means of transmission, ecology and ways of preventing crop losses. In my view, part of the penultimate and all of the final chapter should have been omitted and the space used to expand those chapters which seem unduly

compressed—for example, 'Effect of Viruses on Plants'; the subjects in question are 'Viruses of Animals' and 'Plant Pathogens Confused With Viruses'.

The production is excellent (as befits its price), with clear type and illustrations and agreeably few typographical errors. I regret the absence of titles from the references, making it laborious to check that all are worth inclusion; one suspects that the authors' assurance that, in spite of their selection having been "somewhat arbitrary, most important papers are included," refers mainly to those by themselves and co-workers; otherwise the choice might best be described as whimsical.

The authors have disregarded their own warning in at least one instance. They rightly stress the hazards of identifying viruses on inadequate data, yet they apply the name "swollen shoot" to a virus of cacao trees in Trinidad. To the best of my knowledge, this and the West African swollen shoot virus have no established affinities besides a common vector.

Unquestionably this book is a valuable addition to the few on plant viruses and will be welcomed especially by the small band who teach the subject at English-speaking universities.

A. F. Posnette

Islamic science

Islamic Science: An Illustrated Study.
By Seyyed Hossein Nasr. Pp. xiv+273.
(World of Islam Festival: London,
1976.) £12.50.

A HANDSOME book, with 135 colour plates and 94 black-and-white pictures and diagrams, the photographs mostly by Roland Michaud. As for Seyyed Hossein Nasr's text, it may be recommended to those who already know enough about the subject to make allowances for the author's lack of objectivity and not to be led astray by his inaccuracies and his Humpty-Dumptyish way with words. Many of the author's convictions are shared by all reasonable people. But what the reader must bear in mind is that, although the implied contrast throughout is between the Islamic and non-Islamic worlds, what the author is really contrasting is the modern virtually world-wide technological civilisation on the one hand and an idealised Islamic Golden Age on the other. "In contrast to the modern situation, in traditional technology, of which many examples can still be seen in the Islamic world, the maximum possible recycling of all materials has always been the rule rather than the exception . . . The use of every part of the sheep for purposes as far apart as feeding the family to providing fertiliser for the land to making strings for musical instruments is a case in point" (p234). A case in point indeed; it was no less true in eleventh-century Birmingham than in eleventh-century Baghdad.



Photo: Roland Michaud

Contemporary Persian alchemist at work

The central thesis of the book seems to be summed up on p150: "Islamic civilisation had the means to make complicated machines and apply them to the problems of the daily life of the Islamic community. But like the Chinese, who made gunpowder but never made guns, the Muslims never took that step which would mean the creation of a technology out of harmony with the natural environment." The comparison with China, by the way, would be more acceptable if the previous page were not devoted to illus-

trations from Muslim works on the manufacture of cannon. Medieval Muslim men of science did not invent the steam-engine or the dynamo; nor did their Christian contemporaries, and no discredit to either party for that. But can one ascribe this failure to a conscious self-denying ordinance? It seems an affront to the genius of such men as al-Biruni and Ibn al-Haytham to suggest that they were less willing than any Western savant "To follow knowledge like a sinking star, Beyond the utmost bound of human thought."

As for the other faults alluded to above, one example of each must suffice. "In Islam animals are never 'slaughtered'; they are only allowed to be killed ritually (*dhibh*)" (p244; for *dhibh*, incidentally, read *dhabh*). This will be about as meaningful to most readers as saying "In France people never 'drive' cars; they only conduisent them".

"In Islamic sources the one Hermes of Alexandrian sources became three, hence the term Hermes Trismegistos (from the Arabic *al-muthallath bi'l-hikmah*) . . ." (p198). As the term was in use in the early third century, 400 yr before the coming of Islam, either Dr Nasr is wrong or someone in the Hellenistic world deserves credit for a remarkable piece of intelligent anticipation.

One feels a little mean at criticising so urbane a guide to Cloud-cuckoo-land; with caution on the part of the reader, this is a useful book. Even without, it is very beautiful.

G. L. Lewis

Petroleum geology of North American continental shelves

Canada's Continental Margins and Offshore Petroleum Exploration. (CSPG Memoir No. 4.) Edited by C. J. Yorath, E. R. Parker and D. J. Glass. Pp.898. (Canadian Society of Petroleum Geologists: Calgary, Alberta, 1975.) \$19.

THIS excellent book is unfortunately misnamed, and may be ignored by European geologists (and especially petroleum geologists), for whom it may be very useful. Essentially this volume describes the geology of the continental shelves of North America, from Vancouver on the west coast to Florida on the east coast, of the Arctic Shelves adjacent to Canada, Greenland, Svalbard and Russia, and of the Eastern Atlantic shelves from North Cape to Morocco. These areas are described on the basis of geophysical maps, seabed sampling, sparse well control and

adjacent onshore geology. These data are interpreted in the light of plate tectonics theory, and most papers review the petroleum prospects in the areas concerned.

The volume contains 50 papers which were presented at two symposia: the *Offshore Symposium* held at St John's, Newfoundland in May 1974, and in Calgary, Alberta in September of the same year. The editors took the view that it was more important to publish the book in haste, with minimal editing, rather than to delay publication for careful editing and preparation. This was probably the correct decision. A page of errata is included; there seem to be few misprints. Because of this haste there are maps without scales (for example, p301, 302, 304, 309), metres and feet get used for vertical depths on facing pages in the same paper (p570, 571), some micro-photographs have no scale (p626), some ornaments are not explained in keys (p651), and some gravity maps lack contour values (p304). Although the

editors claim to have checked references for ease of retrieval, I defy anyone to track down those listed on p852.

Much of the material is new, at least to this reviewer. Some of the North American east coast data have been published previously in the bulletins of the American Association of Petroleum Geologists, and even of the Canadian Society of Petroleum Geologists itself. Similarly the material presented on the continental shelves of north-western Europe will be familiar to those who have read *Petroleum and the Continental Shelf of North-west Europe*, edited by A. W. Woodland (for review, see *Nature*, 259, 607; 1976).

On balance, however, the editors are to be congratulated for having collated so much data on such a wide area from so many sources. This volume will provide a valuable source book for geologists, and especially petroleum geologists who are interested in the continental shelves of North America, the Arctic and Europe.

R. C. Selley

obituary

David M. Dennison died on April 3, 1976 at his home in Ann Arbor, Michigan. He was born on April 26, 1900 in Oberlin, Ohio, and, except for various leaves of absence, spent his whole academic career at the University of Michigan, where he joined the faculty in 1927. He was chairman of its Physics Department from 1955 to 1965 and retired in 1971. He was a member of the National Academy of Sciences, a Fellow of the American Physical Society and the recipient of various honours.

With his death, the world of Physics loses again one of that small number of pioneer theorists who made early basic contributions to quantum mechanics and to the understanding of the structure of matter, contributions which today are regarded as self-evident. Dennison obtained his Ph.D. in 1924 from the University of Michigan, where Harrison M. Randall and coworkers were using powerful new techniques for the observation of the infrared spectra of molecules. Dennison then spent three years visiting various physics centres in Europe. In 1926, when he was in Cambridge studying with R. H. Fowler, he proved that the hydrogen nucleus has a spin whose magnitude was half that of a quantum of rotation. That this nucleus might have a spin had been talked about widely, but no evidence had been presented. It is the more remarkable that Dennison's proof did not come from spectroscopic data, but from the study of a baffling anomaly in the variation with temperature of the specific heat of hydrogen. Dennison showed that the two configurations of the hydrogen molecule, one with parallel, the other with opposite nuclear spins, must be considered as two separate gases and that transitions from one into the other are very rare. This

gave a complete quantitative explanation of the specific heat anomaly as well as a clarification of spectral data. That the volume ratio of the two gases had to be taken as three to one determined the value of the spin. The recognition of these two forms of hydrogen has today found an important use in the long-term storage of liquid hydrogen.

Another remarkable contribution made by Dennison in 1932 concerns the ammonium molecule NH_3 . In this molecule the nitrogen atom is in the centre of the triangle, but just outside the plane, formed by the three hydrogen atoms. Dennison noted that a quantum mechanical effect makes it possible for the nitrogen to 'leak' through to the other side. The details were worked out in collaboration with G. E. Uhlenbeck, also at Michigan. It was shown that this non-classical effect caused the molecule to absorb radiation of ~ 1.5 -cm wavelength, which might be produced by miniature magnetrons. Dennison inspired N. H. Williams and C. E. Cleeton at Michigan to attempt a unique experiment to detect this absorption. It was successful and represents the first experiment in microwave spectroscopy. During the second world war, Dennison collaborated with a group developing proximity fuses. Afterwards he spent some time together with T. H. Berlin computing orbits for the Michigan electron accelerator, though his primary interest remained the quantum theory of molecular structure and the interpretation of their spectra to which he contributed many important advances.

David Dennison was an excellent teacher with a genuine concern for students. He was a modest person, who never lost his temper and had many interests outside the field of physics.

He was a fine scholar, a helpful friend with whom it was a great and beneficial pleasure to discuss all sorts of problems.

Samuel A. Goudsmit

Hans Thirring, Professor emeritus at Vienna University, died on March 22, 1976. He held the chair of Theoretical Physics from 1927 to 1958, except during the time of the Nazi regime when he was dismissed because of his pacifist convictions.

Thirring's scientific work dealt mainly with problems in general relativity. The prediction of the 'Thirring-Lense effect' is widely known. He also made important contributions to the quantum theory of specific heats. His 3-yr course on theoretical physics was an excellent example of how to present complicated material in a crystal clear way, and many generations of young physicists profited greatly from his outstanding teaching.

During World War 2 Thirring's thinking concentrated on the question how to achieve a peaceful world. The horrific prospects of nuclear warfare stimulated his activities. Thirring was the first to predict openly the feasibility of a hydrogen bomb, in his book *Die Geschichte der Atombombe* (1946). His further book, *Homo Sapiens*, outlined an educational and psychological way to a world without aggression, hatred and war. He was realist enough to expect not too much effect on mankind from the written word alone, and he put his convictions into practice. He was one of the pioneers of the Pugwash movement and he considered this free community of scientists of high reputation from all nations as one of the few hopeful developments towards peace in his time and for the future.

P. Weinzierl

announcements

Appointments

Professor **Frans E. Wickman** has been elected President of the Royal Swedish Academy of Sciences.

Dr A. Bull has been appointed to the second Chair in Applied Biology at the University of Wales, Cardiff.

Dr Ernest Ambler has been appointed the new Director of the National Bureau of Standards.

Professor Robert F. Whelan has been appointed Vice-Chancellor of the University of Liverpool.

Professor Francois Gros has been appointed as Director of the Institut

Pasteur.

Dr Donald D. Brown will become the Director of the Carnegie Institution of Washington's Department of Embryology to succeed **Dr James D. Ebert** who is moving to the Marine Biological Laboratory in Woods Hole as the full-time President and Director.

Awards

The German Pharmacological Society has given the Schmiedeberg-Plakette to **Dr Hans Kosterlitz** of the University of Aberdeen for his work on opiate receptors.

Sir Karl Popper has been elected a Fellow of the Royal Society.

The IMO Prize has been awarded to **Academician Evgeny K. Fedorov** of the Institute of Applied Geophysics of the Academy of Sciences of the USSR.

Sir James Lighthill and **Dr Max Perutz** have been elected associate members of the French Academy.

Meetings

August 18–21, **Convention of the Western Amateur Astronomers**, San Diego (PAA Reservations, PO Box 505, San Marcos, California 92069).

August 30–September 2, **The Physics of X-Ray Spectra**, Gaithersburg, Maryland (Dr W. G. Schweitzer, Physics Building, Room B164, NBS, Washington DC 20234).

August 30–September 3, **4th Symposium on Recent and Fossil Marine Diatoms**, Oslo (Dr G. R. Hasle, Department of Marine Biology and Limnology, University of Oslo, PO Box 1069 Blindern, Oslo 3, Norway).

September 5–10, **Cell Biology**, Boston (The Secretary, First International Congress on Cell Biology, PO Drawer ICCB, Boulder, Colorado 80302).

September 6–8, **5th European Drosophila Research Conference**, Louvain-La-Neuve (Professor F. A. Lints, Laboratoire de Génétique, Université de Louvain, Place Croix du Sud 2, B-1348 Louvain-La-Neuve, Belgium).

September 6–8, **Lipid Biochemistry**, Paris (J. Polonovski, Faculté de Médecine Saint-Antoine, 27 rue Chaligny, 75571 Paris Cedex 12, France).

September 6–10, **Soil Organic Matter**, Braunschweig, FRG9 (The IAEA, Kärtner Ring 11, PO Box 590, A-1011 Vienna, Austria).

September 13–24, **Oceanography**, Edinburgh (The Organising Committee, Joint Oceanographic Assembly 1976, c/o The Royal Society of Edinburgh, 22 George Street, Edinburgh EH2 2PQ, UK).

September 14–16, **Powder Coating**, Cincinnati (AFP/SME, 20501 Ford Road, PO Box 930, Dearborn, Michigan 48128).

September 14–17, **Nutrition**, Munich (Dr Angelika Janssen, Organisation Secretary, 2nd European Nutrition Conference, Medizinische Poliklinik

der Universität München, Pettenkoferstrasse 8a, D 8000 München 2, FRG). September 14–17, **3rd Symposium on Pharmacology of Thermoregulation**, Banff, Alberta, Canada (Prof. K. E. Cooper, Division of Medical Physiology, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 1N4).

September 15, **DNA Repair and Late Effects**, Vienna (Institut für Biologie, Forschungszentrum Seibersdorf, A-244 Seibersdorf, Austria).

September 19–22, **Gallium Arsenide and Related Compounds**, Edinburgh (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

September 20–24, **Thermal Analysis**, Salford (Dr D. Dollimore, Department of Physical Chemistry, University of Salford, Salford M5 4WT, UK).

September 22–24, **Turbulence in Liquids**, Rolla, Missouri (Turbulence Symposium, Extension Division, University of Missouri-Rolla, Rolla, Missouri 65401).

September 23–24, **Symposium on Ecological Effects of Pesticides**, London (Institute of Biology, 41 Queen's Gate, London SW7 5HU).

September 24–26, **Instrumentation for Synchrotron Radiation**, Daresbury (Mrs S. A. Lowndes, Daresbury Laboratory, Warrington WA4 4AD, UK).

September 27–29, **Wetting, Spreading and Adhesion**, Loughborough (Dr R. J. Akers, Department of Chemical Engineering, University of Technology, Loughborough, Leicestershire LE11 3TU, UK).

October 3–9, **Hill Land**, Morgantown (R. L. Reid, Hill Land Symposium Committee, College of Agriculture and Forestry, West Virginia University, Morgantown, West Virginia 26506).

December 2–9, **Pharmacology**, Buenos Aires (VI Congreso Latinoamericano de Farmacología, Junin 956-5° piso, Buenos Aires, Argentina).

March 7–11, 1977, **Tropical Ecology**, Panama (Dr Reina T. de Araúz, President, Organising Committee, IV International Symposium of Tropical Ecology, PO Box 662, Panama 1, Rep. of Panama).

March 7–11, 1977, **Biologically Active Polypeptides**, Montbello, Quebec (CBC-CIC 1977 Joint Symposium, 151 Slater, Suite 906, Ottawa, Ontario K1P 5H3, Canada).

March 30–April 1, 1977, **Cybernetics of Movement and Classification**, Munich (Univ.-Doz. Dr G. Hauske, Lehrstuhl für Nachrichtentechnik der Technischen Universität München, Arcisstrasse 21, D-8000 München 2, FRG).

April 24–30, 1977, **Radiation Protection**, Paris (Mr Gilbert Bresson,

General Secretary, IRPA IVth International Congress, B.P.33, 92260 Fontenay-aux-Roses, France).

May 2–5, 1977, **Cytology**, Tokyo (A. Meisels, MD, FIAC, General Secretary, 6th International Congress of Cytology, 1050 Chemin Ste-Foy, Quebec 6, P.Q., Canada).

June 19–25, 1977, **Inborn Errors of Metabolism in Man**, Tel Aviv (Scientific Secretariat, Dr O. Sperling, PO Box 16271, Tel Aviv, Israel).

June 27–July 1, 1977, **Amorphous and Liquid Semiconductors**, Edinburgh (Conference Secretariat, Centre for Industrial Consultancy and Liaison, University of Edinburgh, 14 George Square, Edinburgh EH8 9JZ).

July 3–8, 1977, **Immunology**, Sydney (The Secretary General, 3rd International Congress of Immunology, PO Box 391, Darlinghurst, N.S.W. 2010, Australia).

July 11–14, 1977, **Dendrochronology in Northern Europe**, London (The Organising Secretary, Symposium, National Maritime Museum, Greenwich, London SE10 9NF).

August 7–13, 1977, **General Relativity and Gravitation**, Waterloo, Ontario (GR8, Applied Mathematics Department, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada).

September 5–8, 1977, **New Processes of Waste Water Treatment and Recovery**, London (Deadline for abstracts: December 31), (Dr D. H. Sharp, General Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS, UK).

September 5–9, 1977, **Hypoxic Cell Sensitisers in Radiobiology and Radiotherapy**, Cambridge (Dr G. E. Adams, CRC Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN, UK).

September 6–11, 1977, **11th World Congress of Neurology**, Amsterdam (Secretariat, c/o Holland Organising Centre, 16 Lange Voorhout, The Hague, The Netherlands).

September 13–16, 1977, **Nuclear Quadrupole Resonance Spectroscopy**, Osaka, Japan (4th International NQR Symposium, Faculty of Science, Osaka University, Toyonaka 560, Japan).

Autumn, 1977, **Phosphate Compounds**, Rabat, Morocco (Mr M. Kabbaj, Director of Technical Research, Institut Mondial du Phosphate, 8 rue de Penthievre, 75008 Paris, France).

Reports and Publications

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Food Research: Report of Research 1974/1975. Pp. 115. (Sydney: CSIRO, 1975.) [113]

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United States Department of the Interior: Geological Survey. Bulletin 1401: Rapid Analysis of Silicate, Carbonate, and Phosphate Rocks—Revised Edition. By Leonard Shapiro. Pp. v + 76. Bulletin 1417: Annotated Bibliography of Studies on the Density and other Volumetric Properties for Major Components in Geothermal Waters, 1928-74. By R. W. Potter, II, D. R. Shaw and J. L. Haas, Jr. Pp. iii + 78. Water-Supply Paper 1798-L: Effects of Land Use and Retention Practices on Sediment Yields in the Stony Brook Basin, New Jersey. By Lawrence J. Mansue and Peter W. Anderson. Pp. iii + 33. 55 cents. Water-Supply Paper 2039-A: Chemical Quality and Temperature of Water in Flaming Gorge Reservoir, Wyoming and Utah, and the Effect of the Reservoir on the Green River. By E. L. Bolke and K. M. Waddell. Pp. iv + 26 + plate 1. (Washington, DC: US Government Printing Office, 1973, 1974 and 1975.) [183]

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United States Department of the Interior: Geological Survey. Bulletin 1408: New and Refined Methods of Trace Analysis Useful in Geochemical Exploration. By F. N. Ward. Pp. iv + 105. (Washington, DC: US Government Printing Office, 1975.) [193]

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Smithsonian Contributions to Zoology, No. 217: Cyclopoid Copepoda Associated with Asteroid Echinoderms in New Caledonia. By Arthur G. Humes. Pp. iii + 19. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [223]

Royal Netherlands Meteorological Institute. Publication No. 153: On Earthquake Risk for Nuclear Power Plants. (Proceedings of the ESC Symposium in Luxembourg on 20-22 October 1975.) Edited by A. R. Ritsema. Pp. 192. (De Bilt, Netherlands: Royal Netherlands Meteorological Institute, 1976.) [223]

Settlement in Israel. (Israel National Report to Habitat, United Nations Conference on Human Settlements.) Edited by Valerie Brachya and Paulette Mandelbaum. (Publication No. 76-01). Pp. 79. (Jerusalem: Ministry of the Interior, Environmental Protection Service, 1976.) [253]

The Buffaloes of China. By W. Ross Cockrill. (Australian Freedom from Hunger Campaign.) Pp. x + 96. (Rome: FAO; London: HMSO, 1976.) \$8. [253]

United States Department of the Interior: Geological Survey. Professional Paper 801: Jurassic Paleobiogeography of Alaska. By Ralph W. Imlay and Robert L. Determan. Pp. iii + 34. (Washington, DC: US Government Printing Office, 1973.) 65 cents. [253]

Person to Person

Scientist going on sabbatical from Jerusalem would like to exchange flats with someone in London. Four-room flat (3 bedrooms), central heating and all conveniences with schools nearby near the Hebrew University (Beit Hakerem). Available mid-August 1976 for 12 months. (Dr S. Moshkovitz, Geological Survey, 30 Malkhei Street, Jerusalem, Israel.)

NASA is seeking proposals for scientific investigations of Jupiter, to make *in situ* (by probe) and remote measurements (by orbiter) of the planet, its environment and its satellites. Primary emphasis is on direct entry measurements and the characterisation of the magnetosphere of the planet and its interaction with the satellites. Proposals for the Probe investigations by November 1, 1976, and for the Orbiter investigations, December 1, 1976 to Dr M. A. Mitz, Chief, Advance Planning, Lunar and Planetary Programs Office, Code SL, NASA Headquarters, Washington, DC, 20546.

A seminar on Scientific and Technical Publishing in a Multilingual Society is being held in Luxembourg by the Commission of the European Communities on November 11-12, 1976. The chief aim is to generate proposals for measures which might be taken in the framework of the European Community. For further information contact Mr J. M. Gibb, D.G. XIII, European Centre, Kirchberg, Luxembourg.

The David Anderson-Berry prize will be awarded for recent work on the effects of X-rays and other forms of radiation on living tissues.

Published work will be taken into consideration if submitted with the application. In addition to direct application for the Prize, proposals may be made on behalf of others. Applications and proposals must be in the hands of the General Secretary, Royal Society of Edinburgh, 22/24 George Street, Edinburgh EH2 2PQ, Scotland, not later than September 30, 1976.

The Bigfoot Research Society is looking for organisations willing and able to analyse specimens hoped to be collected during field studies of this shy animal. Write to: James R. Spink, Bigfoot Research Society, PO Box 61-1564, North Miami, Florida 33161.

Sydney-Paris. Wanted, two- to three-bedroom furnished flat or house within commuting distance of Meudon-Bellevue (Paris) in exchange for a three-bedroom furnished house in Lindfield (13 km N of Sydney) for approximately 1 yr beginning during September 1976. Dr D. M. Eagles, National Measurement Laboratory, CSIRO, Chippendale, NSW 2008, Australia.

ACCOMMODATION: Scientist going on sabbatical leave to Orsay offers exchange of comfortable three-bedroom house in Peterborough, Ontario, Canada for a similar one in the southern or south-eastern suburbs of Paris from September 1, 1976 to August 31, 1977. Please contact Professor R. Alfred, Physics Department, Trent University, Peterborough, Ontario, Canada.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Twenty-Two Dimensions of the Population Problem. By Lester R. Brown, Patricia L. McGrath and Brice Stokes. (Worldwatch Paper No. 5.) Pp. 83. (Washington, DC: Worldwatch Institute, 1976 Massachusetts Avenue, NW, 1976.) \$2. [263]

Journal of Membrane Science, Vol. 1, No. 1, March 1976. Edited by H. K. Lonsdale. Pp. 1-114. Published Quarterly. Subscription rate: US\$56.95; Dfl. 142. (Amsterdam: Elsevier Scientific Publishing Company, 1976.) [293]

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Introducing WHO. Pp. 88. (Geneva: WHO; London: HMSO, 1976.) Sw. fr. 10. [293]

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Académie Royale de Belgique. Mémoires de la Classe des Sciences. 2e Serie, T. CLII, Fasc. 1: Structures Presque Complexes Généralisées Equations à Structure Presque Complexe. Par Jean-Claude Vansnick. Pp. 46. (Bruxelles: Académie Royale de Belgique, 1975.) [293]

Argentino: Dirección Nacional del Antártico. Contribuciones. No. 169: Características Teratológicas

en Coscinodiscus Bouvet Karst en Puerto Paraiso y Estrecho de Bismarck, Antártida. Por L. J. C. Martínez Macdonald. Pp. 9. No. 171: Estructura y Citología Plineal en Pingüinos Antárticos. Estudio Comparativo en dos Especies (Pygoscelis Papua y Adeliae). Por L. G. A. Benelbaz y Dr. Ramon S. Piezzi. Pp. 15. No. 172: Rasgos Morfológicos de la Isla Deception, Islas Shetland del sur Antártida Argentina. Por Antonio P. Igarzabal. Pp. 30. No. 173: Geología del Extremo Occidental del Cabo Spring—Costa de Danco, Antártida Argentina. Por R. A. Llorente, J. E. Menda y J. P. Spikermann. Pp. 19. No. 174: Estado Volcánico de la Isla Deception, Islas Shetland del Sur, Antártida Argentina. Por J. G. Viramonte y Nestor H. Fourcade. Pp. 12. No. 182: Caracterización de los Estados de Vigilia y Sueño de *Phalacrocorax atriceps* (Aves, Phalacrocoracidae). Por A. P. Tomo, J. S. Pariza y J. M. Affani. Pp. 14. No. 183: El Sistema Inmuno-globulinico del *Pygoscelis Papua*. Por O. D. Castrelos, M. Romero Mercado, C. Abatangelo y R. A. Margni. Pp. 12. (Buenos Aires: Instituto Antártico Argentino, 1973 and 1974.) [303]

Smithsonian Contributions to Zoology. No. 216: Study of the Monotypic Fish Family Pholidichthyidae (Perciformes). By Victor G. Springer and Warren C. Frehofer. Pp. iii + 43. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [303]